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REVIEW

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Jonathan H. Aron · Christopher L. Bowlus

Abstract Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease histologically characterized by the presence of intrahepatic and/or extrahepatic biliary duct concentric, obliterative fibrosis, eventually leading to cirrhosis. Approximately 75% of patients with PSC have inflammatory bowel disease. The male predominance of PSC, the lack of a defined, pathogenic autoantigen, and the potential role of the innate immune system suggest that it may be due to dysregulation of immunity rather than a classic autoimmune disease. However, PSC is associated with several classic autoimmune diseases, and the strongest genetic link to PSC identified to date is with the human leukocyte antigen *DRB01*03* haplotype. The precise immunopathogenesis of PSC is largely unknown but likely involves activation of the innate immune system by bacterial components delivered to the liver via the portal vein. Induction of adhesion molecules and chemokines leads to the recruitment of intestinal lymphocytes. Bile duct injury results from the sustained inflammation and production of inflammatory cytokines. Biliary strictures may cause further damage as a result of bile stasis and recurrent secondary bacterial cholangitis. Currently, there is no effective therapy for PSC and developing a rational therapeutic strategy demands a better understanding of the disease.

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Abbreviations

PSC	Primary sclerosing cholangitis
IBD	Inflammatory bowel disease
HLA	Human leukocyte antigen
UC	Ulcerative colitis
Mdr2	Multidrug resistance-2
VCAM-1	Vascular cellular adhesion molecule-1
PG-PS	Peptidoglycan–polysaccharide
DNBS	Dinitrobenezenesulfonic acid
TNBS	Trinitrobenzene sulfonic acid
DSS	Dextran sulfate sodium
α-GalCer	α-Galactosylceramide
NK	Natural killer
KIRs	Killer immunoglobulin-like receptors
ITIMs	Immunoreceptor tyrosine-based inhibitory motifs
ITAMs	Immunoreceptor tyrosine-based activating motifs
MIC	Major histocompatibility complex class I chain-related
CCR5	CC-type chemokine receptor 5
TCR	T-cell receptor
MAdCAM-1	Mucosal adressin cell adhesion molecule-1
CLA	Cutaneous lymphocyte antigen
AIH	Autoimmune hepatitis
PBC	Primary biliary cirrhosis
LIL	Liver-infiltrating lymphocytes
VAP-1	Vascular adhesion protein-1
HEV	High endothelial venules
TGF	Transforming growth factor
NOS2	Nitric oxide synthase 2
p-ANCA	Perinuclear anti-neutrophil antibodies

BPI	Bactericidal/permeability increasing
BEC	Biliary epithelial cells
PAMPs	Pathogen-associated molecular patterns
LPS	Lipopolysaccharide
TLRs	Toll-like receptors

Introduction

Primary sclerosing cholangitis (PSC) is a chronic chole-static liver disease characterized by concentric and obliter-ative fibrosis of the intrahepatic and/or extrahepatic bile ducts and a lymphocytic portal tract inflammation leading to cholestasis, cholangitis, and cirrhosis [1, 2]. In addition to liver disease, 75–90% of PSC patients have either a history of or co-existent inflammatory bowel disease (IBD), primarily ulcerative colitis (UC) [2, 3]. Interestingly, unlike IBD which has a slight female predominance, PSC has a 2:1 male predominance. Although the disease may affect children and older adults, the median age of onset is in the fourth decade [4]. The natural history of the disease is quite variable with an average time from diagnosis to death or liver transplant of 12 to 15 years. In addition to cirrhosis, approximately 10–15% of PSC patients will develop cholangiocarcinoma during their lifetime [3]. Short of liver transplantation, no therapy has been shown to alter the natural history of PSC. Herein we review the current understanding of the immunobiology of PSC with partic-ular attention to the epidemiologic, genetic, and functional characteristics of this disease.

Epidemiology

Epidemiologic studies of PSC are challenging because the disease is rare, often requires specialized expertise, and until recently, could only be diagnosed by invasive procedures. Therefore, population-based studies likely underestimate the true prevalence and incidence of PSC. Nevertheless, the incidence (0.9–1.3 per 100,000/year) and prevalence (8.5–14.2 per 100,000) of PSC have been reported to be similar in Oslo, Norway, Wales, and Olmstead County, Minnesota [5–8]. However, in the largest study published to date, the incidence and prevalence were considerably lower in a general medical practice database of the UK [9]. There appears to be a lower prevalence in Southern Europe, Asia, and Alaska [10–12].

While the male to female predominance of 2:1 for PSC has been largely reported, a study from Turkey reported that 15/16 PSC patients in their series were women [13] and a Canadian study reported a slight male predominance that did not reach statistical significance, with an age-adjusted incidence rate of 1.01 per 100,000 person-years for men versus 0.84 per 100,000 person-years for women [8]. Age at diagnosis has also varied by geographic region. While the majority of patients have been reported to be diagnosed in their thirties [14], studies from Japan note a bimodal age distribution with most diagnoses occurring in the third decade and a second peak in the seventh decade of life. Within this distribution, younger patients were more likely to have IBD than older patients and older patients were more likely to have autoimmune pancre-atitis and cholangiocarcinoma [15, 16]. The average age of patients diagnosed with cholangiocarcinoma in the Japan study was 58. The overall incidence of cholangio-carcinoma reported in this population was 3.6%, much lower than the 10–15% that has been reported in previous studies [3, 15].

The strength of the association of PSC with IBD also differs by geographic origin. Approximately 80% of PSC patients from northern Europe and the USA have concom-itant IBD [17–20], while only 50% from Southern Europe [12, 21] and 35% from Asia have IBD [11, 22, 23]. The majority of PSC patients with IBD have ulcerative colitis although Kaplan et al. reported that 38.8% of patients with PSC in Canada have Crohn's disease versus only 5.7–13.6% reported in other studies [8]. To explain this discrepancy, they noted that the prevalence of CD in Alberta was 1.5 times the prevalence of UC, whereas UC is more prevalent than CD in the rest of the world. They hypothesized that the increased rates of CD seen among PSC patients may reflect an unexplained occurrence of rising incidence of CD in Canada and worldwide [8]. Alternatively, the IBD associated with PSC often shares features with both UC and CD [24].

Immunopathogenesis of PSC

The association of PSC with IBD suggests that like the latter, PSC is not necessarily a classic autoimmune disease in the sense that there is targeted destruction of tissue directed at a specific self-antigen. Rather, IBD is the result of an abnormal innate immune response to antigens of the intestinal flora, which activates an adaptive immune response [25]. Genome-wide association studies and sub-sequent functional studies have implicated several genes such as NOD2 and ATG16L1, both involved in the intracellular processing of bacterial antigens [26–28]. In the case of CD, this leads to a predominantly T_H-1 type of immune response and increases in IL-17-producing lym-phocytes. In contrast, ulcerative colitis tends to be more of a T_H-2 response. Whether PSC, which tends to be characterized by T_H-1 cytokines and stricturing reminiscent of CD, involves similar mechanisms has not been fully

investigated. Further complicating the matter is the association of PSC with (1) classic autoimmune diseases such as type I diabetes, thyroid disease, rheumatoid arthritis, and others; (2) the human leukocyte antigen (HLA)-DRB1*0301 haplotype; and (3) autoantigens to neutrophils and biliary epithelial cells.

Several working models have been proposed to explain many of the peculiar features of PSC, namely, the strong association with IBD, the observation that PSC may develop after total colectomy and that the liver disease activity of PSC does not correlate with the intestinal disease activity of IBD [1, 29]. In fact, IBD is often quiescent even in the most severe cases of PSC. Notably, the IBD associated with PSC, whether classified as UC or CD, almost invariably affects the entire colon. In addition, rectal sparing and involvement of the ileum are more common in UC patients with PSC compared to those without PSC (58% and 51% versus 6% and 7%, respectively) [24].

Although hypotheses related to the etiology of PSC have been generated and several lines of evidence support various aspects of each model, a unifying hypothesis remains to be proposed. Vierling proposed that the immunopathogenesis of PSC involves multiple steps starting with the activation of a cholangiocyte by a bacterial pathogen that translocates to the liver from the gut [30]. Over time, there is chronic inflammation, periductal fibrosis, and ischemic atrophy of biliary epithelia leading to cholestasis, obstructive strictures, and eventually biliary cirrhosis [31].

The second hypothesis addresses how intestinal mucosal lymphocytes home to the liver. This theory suggests that lymphocytes are activated in the bowel of IBD patients and then aberrantly recruited to extraintestinal sites. The prodigious work of Adams et al. supports their hypothesis that a network of adhesion molecules and chemokine receptors that are normally restricted to the gut are aberrantly expressed in the liver leading to the recruitment of intestinal lymphocytes through the enterohepatic circulation [32–37]. However, the mechanisms leading to the aberrant expression of adhesion molecules and chemokines in the liver or even if they are dependent upon liver or intestinal factors remain unknown.

A third theory recently introduced by Fickert et al. compares the pathogenesis of PSC to arteriosclerosis [38]. Bile acids which can induce apoptosis and necrosis of cholangiocytes are normally excreted in mixed micelles with phospholipids and cholesterol to protect cholangiocytes. In the multidrug resistance knockout mouse (Mdr2−/−) which develops a PSC-like liver pathology (discussed in detail below), absence of phospholipids as a consequence of the Mdr2 defects results in bile acid toxicity and also in cholesterol-supersaturated bile, which could facilitate oxidation similar to the process of atherosclerosis.

In addition, cholangiocytes from Mdr2−/− mice express cell adhesion molecules such as vascular cellular adhesion molecule-1. Further similarities in chemokines, growth factors, and cytokines support some commonality between atherosclerosis and PSC. However, support for this theory of toxic bile from humans is lacking. Genetic studies of the human ortholog of Mdr2 (MDR3) have not found any association of genetic variants with PSC susceptibility, and in PSC patients with a normal bilirubin, biliary secretion of bile acids and lipids has previously been shown to be normal [39–41].

Animal models of PSC

Given the abundance and variety of animal models from rodents to primates with spontaneous and induced inflammatory bowel diseases, it is surprising that none of these animal models have been reported to recapitulate all of the features of PSC (Table 1). Whether there is a unique characteristic of the human liver that is not shared by these model organisms or there has been a lack of systematic phenotyping of IBD models is unclear.

In addition to colitis, an ideal animal model of PSC would include histologic evidence of fibrous obliterative cholangitis and cholangiographic evidence of both intra- and extrahepatic duct involvement. Animal models described to date include those involving bacterial cell wall components or colitis; those induced by injury to biliary epithelial cells or endothelial cells of hepatic arterioles or peribiliary capillaries; and those induced by toxic, infectious, or intraluminal injury of the biliary tract [42].

Bacterial components

A potential link between bacterial components and hepatobiliary inflammation was first substantiated by Chadwick and colleagues who demonstrated that N-formylated chemotactic peptides that are produced by several species of intestinal bacteria undergo enterohepatic circulation and that the level of these compounds is increased in experimental colitis [43–45]. In addition, rectal administration of N-formyl L-methionine L-leucine L-tyrosine in rats with acetate-induced colitis results in a biliary-based inflammation consisting of macrophages and neutrophils in the early stages and subsequently CD4+ and CD8+ T cells [46, 47].

In a series of experiments with surgically created self-filling jejunal blind loops leading to bacterial overgrowth, Lichtman and colleagues demonstrated that in genetically susceptible rat strains hepatobiliary injury with features similar to PSC develops including bile duct proliferation, fibrosis, and acute and chronic periportal and focal

Table 1 Animal models of primary sclerosing cholangitis

Animal	Treatment	IBD	Portal Inflammation	Biliary strictures
Rat [47]	N-formyl L-methionine L-leucine L-tyrosine	Yes (acetate-induced)	Macrophages/neutrophils (early) T cells (late)	Unknown
Rat [48–50, 133]	Self-filling jejunal blind loops	No	Yes	Fibrosis, strictures not documented
CD-1 mouse [55]	Dextran sulfate sodium	Yes	Yes	Unknown
Cftr−/− [57]	Dextran sulfate sodium	Yes	Yes	Unknown
SAMP1/YitFc [58]	None	Yes	Yes	Unknown
Mdr2−/− [59]	None	No	Yes	Yes

parenchymal inflammation [48]. The lack of similar findings in self-emptying blind loops that do not develop bacterial overgrowth suggested a role for bacteria or their cell wall components. The role of bacterial peptidoglycans was supported when the effects of the blind loops were abrogated by oral metronidazole and tetracycline or by mutanolysin, a muralytic enzyme which cleaves the beta 1-4 N-acetylmuramyl-N-acetylglucosamine linkage of peptidoglycan–polysaccharide [49, 50]. These studies along with human studies suggesting an increase in intestinal permeability in IBD make plausible a theory of PSC involving the translocation of bacterial cell wall components via the portal circulation to the liver and inducing hepatobiliary inflammation and injury presumably through a pathway that initially involves activation of the innate immune response. However, this model does not have colitis and there is no evidence that bacterial overgrowth in humans leads to PSC. In fact, Bjornsson et al. concluded that small intestinal bacterial overgrowth and increased intestinal permeability are not important in the pathogenesis of chronic PSC based on their results that only one of 22 PSC patients had small intestine bacterial overgrowth and intestinal permeability of their patients did not differ significantly from that of controls [51].

Induced colitis

Several models of spontaneous and induced colitis have been studied to determine the pathologic basis of the association between colitis and PSC. Intrarectal instillation of 2,4-dinitrobenzenesulfonic acid (DNBS) consistently results in colitis in rodents, but there is no evidence of liver injury in this model. In C57BL/6 mice, DNBS treatment has led to P-selectin-dependent recruitment of lymphocytes to post-sinusoidal venules, but this was transient and not associated with an increase in liver enzymes [52]. In IL-10-deficient mice that are prone to spontaneous colitis, DNBS treatment resulted in elevation of ALT and bilirubin which was significantly greater compared to wild-type mice but

this was not associated with any histologic evidence of inflammation [53]. Trinitrobenzene sulfonic acid (TNBS)-induced colitis, which is more similar to Crohn's disease, has failed to result in elevation of liver enzymes or demonstrate any histologic abnormality in the liver [54].

Despite a lack of liver inflammation in these models, the permeability of hepatocyte tight junctions is increased by both DNBS- and TNBS-induced colitis, the former being enhanced by IL-10 deficiency [53, 54]. This is not a direct effect of these agents on the liver as endoportal injection of TNBS does not cause changes in tight junction permeability [54]. These findings leave open the possibility that alteration of hepatocyte tight junctions in response to intestinal inflammation leads to passage of intestinal antigens or bacterial components from the sinusoid across the tight junction and into the canilicular space leading to macrophage and neutrophil recruitment, bile duct inflammation, and injury.

In contrast to DNBS and TNBS, colitis induced in CD-1 mice by dextran sulfate sodium (DSS) in drinking water is associated with histologic changes including cell infiltration around bile duct and focal necrosis [55]. Interestingly, in this model, cholangitis appeared to follow the development of colitis. After 14 days of DSS treatment, nearly all animals developed colitis whereas fewer than 10% developed liver inflammation and only 1/3 of mice developed cholangitis after 28 days. However, even prior to histologic changes, there was an increase in the CD4/CD8 ratio of liver lymphocytes, and analysis of mononuclear cell function revealed high levels of interferon-γ production in contrast to IL-4 and IL-10. In addition, repeated injections of α-galactosylceramide, a ligand for natural killer T (NKT) cells, ameliorated the liver inflammation but had no effect on the colitis [56]. The improvement in liver histology was accompanied by changes in liver mononuclear cells including a reduced CD4/CD8 ratio, reduced percentages of NKT and natural killer (NK) cells, decreased interferon-γ production, and increased IL-4 production. Although this model provides an interesting opportunity to examine the

link between colitis and liver inflammation, it has not been demonstrated to develop bile duct damage or fibrosis.

Given the similarities between PSC and the biliary disease associated with cystic fibrosis, Freedman et al. have investigated the possible role of the gene responsible for cystic fibrosis (*cftr*) in PSC. They demonstrated that while *cftr−/−* mice have normal liver histology, DSS-treated *cftr−/−* mice not only develop colitis but also increased serum alkaline phosphatase and inflammation around the portal tracts including bile duct infiltration by mononuclear cells and bile duct proliferation [57].

Spontaneous models of colitis

SAMP1/YitFc mice develop spontaneous ileitis and have been reported to develop inflammation around intrahepatic and extrahepatic bile ducts [58]. Although expression of the chemokine CCL25 which has been associated with human PSC was not identified in the liver of these mice, further characterization of the liver disease in this model is awaited.

Genetically altered models

One of the more recent mouse models of PSC is the multidrug resistance gene (*Mdr2*) knockout mouse. Targeted disruption of *Mdr2* leads to sclerosing of the biliary tree in mice. *Mdr2−/−* mice develop extra- and intrahepatic biliary strictures and dilations, onion skin-type periductal fibrosis, and focal obliteration of bile ducts similar to that seen with primary and secondary sclerosing cholangitis in humans. Presumably, biliary phospholipids that are normally transported into bile via the canalicular phospholipids flippase Mdr2 and form mixed phospholipid-bile acid micelles protect cholangiocytes from bile acid-induced cell injury. Biliary phospholipids are absent in *Mdr2−/−* which may lead to toxic bile acid-induced damage resulting in sclerosing cholangitis [59]. The portal inflammation in *Mdr2−/−* is unlikely to be of infectious origin as no bacterial translocation has been found in these animals, and bacterial counts and cultures are not different from controls [59]. However, unlike human PSC, the *Mdr2−/−* do not develop IBD [38]. Like the *Cftr−/−* mice, this model points to the potential role of hepatobiliary transporters and changes of bile composition in the pathogenesis of PSC [60].

Genetic factors predisposing to PSC

There is more than an 80-fold increased risk of PSC among first degree relatives suggesting a genetic link to the etiology of PSC [4]. Bergquist et al. reported that among a national Swedish cohort of PSC patients ($n=678$), the risk of cholangitis was increased in offspring, siblings, and parents of the PSC patients, compared with relatives of a control group with hazard ratios of 11.5, 11.1, and 2.3, respectively [61]. Despite this strong genetic risk, the precise genes underlying this susceptibility have not been identified. Candidate genes ranging from mediators of fibrosis to bile acid transporters as well as immune-related genes have been reported. In most cases, the studies are underpowered, and with the exception of the human leukocyte antigen, the results have failed to be replicated in additional cohorts. A comprehensive accounting of the genetics of PSC is beyond the scope of this review. Rather, we will highlight those genetic studies that give insight into the immunopathogenesis of PSC.

HLA and related gene associations

Like many autoimmune diseases, an association with the HLA complex on chromosome 6p21 with PSC has been well documented. The initial reports of an HLA association with PSC came over 20 years ago from Norway and the UK when Schrumpf et al. and Chapman et al. reported a greater prevalence of the HLA class I allele B8 in PSC patients compared to controls [62, 63]. Although similar frequencies of HLA-B8 have been reported in PSC patients from Finland and Australia, no associations with HLA-B8 or other HLA-B alleles were identified when studied in PSC populations from Italy or Brazil [64–67].

The initial report of an association between PSC and HLA class II genes utilized serologic methods and identified DR3 and DR52a as susceptibility markers. The first study from Norway reported that 70% of PSC patients with UC carried the HLA-DR3 antigen [62]. Subsequently, all 29 PSC patients who underwent liver transplant at UCLA were remarkably reported to be DR52a-positive [68]; however, this report was later retracted [69]. Soon after, molecular typing suggested that at least two susceptibility alleles are encoded in the class II HLA, specifically *DRB1*0301* (DR3) and *DRB1*13* (DR6), which are associated with the haplotypes defined by *DRB1*0301, DQA1*0501,DQB1*0201,* and *DRB1*13,DQA1*0103, DQB1*0603* [70, 71]. In fact, approximately half of all PSC patients in Norway and Sweden carry at least one of these two haplotypes compared to less than 20% of the general population. It is important to note, however, that these haplotype associations were not seen in PSC patients from Italy and of the two, only the association with *DRB1*13,DQA1*0103,DQB1*0603* was present in PSC patients from Brazil [64, 65, 71]. In contrast, the *DRB1*04, DQA1*03,DQB1*0302* haplotype (DR4) has been associated with a protective effect in Scandinavia and the UK. Because of the strong linkage disequilibrium within the HLA

region, identifying the gene or genes that account for the associations with these haplotypes has been problematic.

In addition to HLA associations, combinations of HLA class I alleles and killer immunoglobulin-like receptors (KIRs) have been found to affect susceptibility to autoimmune disease and infection. NK cell effector function is balanced by inhibitory and activating receptors [72]. A key set of NK receptors is KIR which bind HLA class I molecules. At least 14 functional KIR genes are present on chromosome 19q13.4 where they exhibit significant allelic and haplotypic variability, the latter of which is largely related to the presence or absence of activating KIR genes. Inhibitory KIRs encode immunoreceptor tyrosine-based inhibitory motifs in their cytoplasmic tails. Activating KIRs interact with DAP12 homodimers that contain immunoreceptor tyrosine-based activating motifs. Genetic studies implicate specific combinations of HLA and KIR alleles in autoimmunity, tumor immunosurveillance, and viral diseases. Combinations expected to increase activation of NK cells are associated with autoimmunity [73], slow progression of human immunodeficiency virus [74], and protection against hepatocellular carcinoma in hepatitis C virus (HCV) infection [75]. In contrast, the combination of HLA-CAsn80 (HLA-C1) and KIR2DL3 has been associated with clearance of HCV [76]. Karlsen et al. investigated the possible interaction between HLA class I alleles and KIR genes in 365 Scandinavian PSC patients and 368 healthy controls [77]. The frequency of HLA-Bw4 and HLA-C2, ligands for the inhibitory KIRs 3DL1 and 2DL1, respectively, was significantly reduced in PSC patients compared with controls suggesting an increase in NK cell activity by decreased inhibition.

In addition to the decreased suppression of NK cell activity through HLA–KIR interactions, genetic evidence suggests a possible role for the direct activation of NK cells through the major histocompatibility complex class I chain-related (MIC) genes. MICA and MICB are encoded with the HLA region and their proteins activate NKG2D receptors on NK cells. Norris et al. found a strong protective effect of the MICA*002 allele with an allele frequency of 0.180 in controls compared to 0.032 and 0.0 in two independently collected cohorts of PSC cases [78]. In contrast, the MICA*008 allele frequency was increased in both sets of cases (0.66 in both) compared to controls (0.48). Although the MICA*008 allele is part of the B8-DR3 haplotype, the association was also observed when the MICA*008 allele was on a B7-DR15 haplotype. In contrast to these UK cohorts, a study of 130 Norwegian PSC patients and 306 healthy controls found associations with the MICA5.1 and MICB24 alleles [79]. However, these alleles are also found on the extended B8-MICA5.1-MICB24-DR3 haplotype and are not independent of HLA-B8 or DR3. Attempts at further dissection of the HLA region have only extended the HLA-DR3-associated haplotype more telomerically

[80]. Future efforts with larger patient cohorts and more densely spaced markers may eventually lead to the identification of the causative variant or variants, but the strong linkage disequilibrium of the region may prove to be an insurmountable barrier.

Candidate genes

With the strong association between PSC and UC, it would not be surprising to find that they shared some common genetic basis. However, this has not proven to be the case so far. Unlike PSC, the association between UC and HLA has been weak and inconsistent. Most consistent in UC are the positive associations with the DRB1*0103 and DRB1*1502 alleles, and a negative association with DRB1*0401. In a direct comparison of 365 Scandinavian PSC patients, 330 Norwegian ulcerative colitis patients, and 368 healthy controls, Karlsen et al. concluded that HLA associations in PSC were of greater impact and mostly distinct from those in UC [81]. Similarly, studies comparing IBD susceptibility genes in PSC and IBD cohorts have failed to show any common genetic links including studies of NOD2/CARD15, TLR-4, CARD4, SLC22A4, SLC22A5, DLG5, and MDR1 [82, 83].

In addition to HLA alleles, ICAM-1 gene polymorphisms have been implicated in UC as well as a number of other inflammatory disorders including multiple sclerosis and Behcet's disease. ICAM-1 mediates leukocyte adhesion during immune responses and is important in transendothelial migration of neutrophils and T-cell activation. Previous studies have demonstrated expression of ICAM on proliferating bile ductules and interlobular bile ducts in PSC patients with advanced disease. The polymorphism K469E in exon 6 leads to a change from glutamic acid to lysine in the Ig-like domain 5 of ICAM-1 which is thought to affect interactions between LFA-1 and B cells. Yang et al. reported that in 104 PSC patients and 213 healthy controls from the UK the E/E frequency of the K469E polymorphism was significantly lower in PSC than in the control population (12% versus 24%, OR 0.41) [84]. However, a larger study of a Scandinavian PSC cohort was not able to reproduce these findings [85].

CC-type chemokine receptor 5 (CCR5) is a cell surface receptor expressed on T cells and macrophages which is activated by several chemokines, including RANTES, MIP1a, and MIP1b. A 32-base pair deletion (CCR5-Δ32) in the CCR5 gene leads to a frame shift and encodes a non-functioning receptor. Studies in several population have reported associations of PSC with the Δ32 polymorphism [86–89]. However, two studies showed a protective effect of the allele, while two others suggested that the allele increased susceptibility. Still, the largest study found no association at all [90].

Cellular immune response in PSC

Teasing out the causative immune response of PSC from the reactive responses to cholangitis, inflammatory bowel disease, and cirrhosis has been a major obstacle in the understanding of PSC. Although the inflammatory infiltrate of PSC is largely comprised of T cells, other cell types including NK cells, macrophages, B cells, and biliary epithelial cells are likely to play important roles in the immunopathogenesis of PSC (Fig. 1).

Innate immune responses

The activation of the innate immune system as a primary inciting event of PSC has been proposed by several

investigators [31]. According to this theory, PSC is triggered by bacteria or more likely, pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), lipoteichoic acid, peptidoglycans, and unmethylated bacterial dinucleotide motifs that enter the portal circulation through an inflamed permeable intestine. PAMPs activate macrophages, dendritic cells, and NK cells through pattern recognition receptors, including Toll-like receptors (TLRs) and CD14 leading to the secretion of cytokines which in turn activate NK cells (IL-12) and promote recruitment and activation of lymphocytes (TNF-α, IL-1β, and CXCL8). NK cells may also be activated by MHC Class I chain-related gene products MICA and MICB, which are stress-induced proteins that can promote
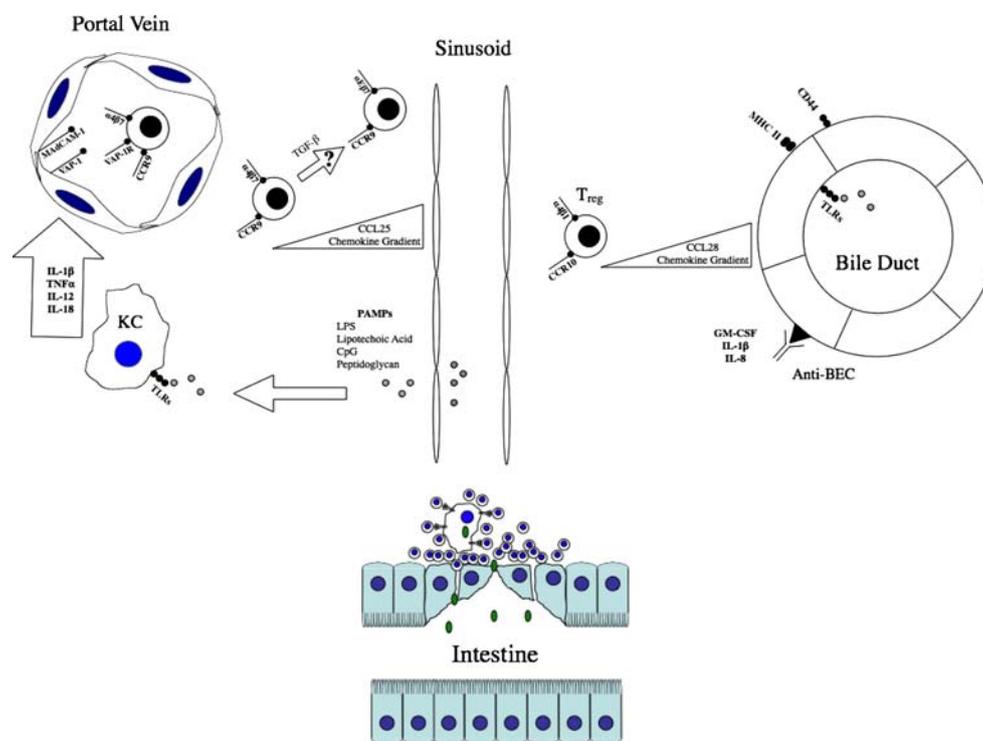
Fig. 1 Proposed mechanism for the immunopathogenesis of primary sclerosing cholangitis. Prior to the development of PSC, intestinal lymphocytes are activated in gut-associated lymph tissue and primed by dendritic cells to express $\alpha 4\beta 7$ and CCR9 which result in the homing of these cells to MAdCAM-1 and CCL25, respectively. Normally, the expression of MAdCAM-1 and CCL25 is restricted to the gut, but in PSC, MADCAM-1 is found on portal vein endothelium and CCL25 on periportal sinusoidal endothelium. Although it has yet to be defined where lymphocytes enter the liver (portal venules, sinusoids, or post-sinusoidal capillaries), the expression of MAdCAM-1 and CCL25 leads to the recruitment of CD44+ $\alpha 4\beta 7$+ CCR9+ memory cells from the gut. The mechanisms leading to the expression of MAdCAM-1 and CCL25 in the liver are unknown but the latter appears to be PSC-specific. In addition, the recurrence of PSC after liver transplantation suggests that this is not an aberrant property inherent in the PSC liver. Induction of MAdCAM-1 and CCL25 may be in response to pathogen-associated molecular patterns (PAMPs) that enter the liver from the gut via the portal vein or sinusoids and bind Toll-like

receptors (TLRs) on macrophages (Kuppfer cells, KC) and dendritic cells. Activation of these cells leads to the secretion of inflammatory cytokines which have been shown to induce MAdCAM-1 expression on hepatic endothelial cells. The reported enrichment of $\alpha E\beta 7$ lymphocytes in the PSC has been suggested to result from a transition of $\alpha 4\beta 7$+ to $\alpha E\beta 7$+ induced by transforming growth factor-β (TGF-β). The localization of lymphocytes to the biliary epithelium is poorly understood. Biliary epithelial cells (BEC) can express a variety of cytokines and chemokines as well as MHC class I and II, CD44, and TLRs. LPS, a potent TLR-4 ligand, induces CCL28 production by BEC leading to the recruitment of $\alpha 4\beta 1$+ CCR10+ regulatory T cells (Treg). However, this phenomenon is seen in a variety of inflammatory liver diseases and is not specific for PSC. We should also note that while TLRs are present on BEC, they are localized to the apical membrane and likely only have contact with PAMPs in the case of ascending cholangitis. Anti-BEC antibodies have been detected in PSC patients and stimulate the production of several cytokines by BEC that could lead to localized recruitment of inflammatory cells

the cytotoxic function of NK, NKT, and γδT cells through the NKG2D receptor.

Similar to the intestinal mucosa, the biliary mucosa expresses multiple TLRs and their expression has been shown to be induced in a variety of liver diseases [91]. Interestingly, IgG directed against biliary epithelial cells (BEC) has been found in the sera of some PSC patients. These sera induce the expression of TLR4 and TLR9 on BEC in culture and can be found to co-localize with these same TLRs on BEC in situ [92]. In fact, treatment of BEC with PSC sera containing anti-BEC antibodies induces secretion of GM-CSF, IL-1β, and IL-8, which in turn may lead to the recruitment of neutrophils, macrophages, and T cells.

However, the targets of these anti-BEC antibodies remain unknown and other evidence that innate immune responses are dysregulated in PSC is largely circumstantial. Histologically, macrophages appear to accumulate in the sinusoidal and perisinusoidal spaces in PSC but not in primary biliary cirrhosis (PBC) and other biliary tract diseases. The accumulation is independent of necrosis, cholestasis, or neutrophil infiltration but whether the increased number of macrophages is a primary cause of PSC or secondary to chronic cholangitis remains to be determined [93].

Further complicating the interpretation of these types of studies are the high rates of gram-negative biliary isolates from patients with dominant stenosis. Compared to those without a dominant stenosis, these PSC patients have a higher load of bacteria and a shorter time to liver transplantation [94]. Other organisms postulated to be involved in the induction of the innate immune response in PSC include *Chlamydia* spp. and *Helicobacter pylori*. Ponsioen et al. found an elevated seroprevalence of *Chlamydia*-LPS antibodies in PSC patients compared to a matched control group. The lack of *Chlamydia* spp. in cultures of bile from these patients suggested that these findings were not due to active infection [95]. Amplification of 16S ribosomal RNA from explanted livers of 25 patients with PSC detected *Helicobacter* sequences in perihilar ductal and liver tissue of six of these patients. However, three of 31 control livers with non-biliary tract disease also demonstrated *Helicobacter* rRNA sequences [96]. Similarly, Boomkens et al. reported no significant difference between the incidence of *Helicobacter* DNA in liver tissue form PSC patients compared to controls [97]. In addition, there has not been any evidence that *Helicobacter* affects the histology of biliary epithelium [98].

Liver T cells

While the lymphocytic infiltrate of PSC has been repeatedly shown to be primarily T cells, the composition of T-cell subsets varies by study. A predominance of CD4+ T cells with a Th-1 phenotype [99] has been shown by some, while others have found a predominance of CD8+ T cells [100, 101] or no difference in the CD4/CD8 ratio compared to other liver diseases [102]. These inconsistencies may be explained by the distribution of T-cell subsets within the liver in which CD4+ T cells predominate the portal infiltrate, while CD8+ T cells comprise the majority of the lobular infiltrate [103]. Thus, PSC patients with more significant lobular inflammation may display a greater percentage of CD8+ T cells.

There is some evidence that the T cells may have oligoclonal restriction. Probert et al. reported that T cells isolated from the common bile duct epithelium obtained during endoscopic retrograde cholangiography of two PSC patients had a large number of CD3+ CD43+ CD45RO+ lymphocytes [104]. Analysis of the variability of the T-cell receptor (TCR), specifically the complimentarity determining region 3 length and TCRVβ usage, suggested that the derived cell lines were oligoclonal. Consistent with these findings, Broome et al. demonstrated that the liver infiltrates of PSC patients more often stain positive for TCRVβ3 compared to those with PBC and healthy controls [105].

In addition to the αβ T cells infiltrating the liver, there are increased proportions of γδ+ T cells in PSC. The percentage of γδ+ T cells in peripheral blood of PSC patients is greater than healthy controls and co-express IL-2 receptor and CD45RO suggesting that they have an activated memory phenotype [106, 107]. An increase in the absolute number and proportion of γδ T cells has also been reported in the portal area of PSC patients [106].

Integrins, chemokines, and chemokine receptors

Tissue-specific recruitment of lymphocytes to inflammation involves the coordinated recognition of "addressins" expressed by vascular endothelial cells by homing receptors on the lymphocyte along with interactions of chemokines and chemokine receptors. For example, lymphocytes activated by dendritic cells in gut-associated lymphatic tissue are programmed to express the α4β7 integrin and the CCR9 chemokine receptor. The ligand for α4β7 is the mucosal adressin cell adhesion molecule-1 (MAdCAM-1) which is specifically expressed on the intestinal endothelium and during inflammation on intestinal mucosa. The CCR9 ligand CCL25, which is also capable of activating α4β7, is expressed preferentially in the intestine as well. The combination of MAdCAM-1 and CCL25 is critical for the specific recruitment of α4β7+CCR9+ lymphocytes to the intestine. Similarly, homing to the skin involves interactions between cutaneous lymphocyte antigen with E-selectin and CCL17 with CCR4. However, recruitment of lymphocytes to the liver does not appear to involve a specific pairing of adhesion molecules and chemokines. Rather, multiple adhesion molecules and chemokines appear to

recruit lymphocytes to the liver. Several of these molecules have been implicated in the pathogenesis of PSC, but their specificity to PSC as opposed to being generic in liver inflammation requires further exploration.

Most notably, MAdCAM-1 was initially thought to be confined to gut endothelium, but has since been shown to be expressed in the portal vein and sinusoidal endothelium in autoimmune-mediated liver disease. Grant et al. reported the presence of MAdCAM-1 staining in the portal veins of 11/16 PSC patients, 6/10 autoimmune hepatitis (AIH) patients and 3/11 PBC patients [36]. Dual-color immuno-fluorescence demonstrated the proximity of $\alpha 4\beta 7$ T cells to MAdCAM-1-positive vessels, and adhesion assays confirmed the functionality of the interaction. Somewhat surprisingly, however, the frequency of $\alpha_E\beta 7+$, but not $\alpha 4\beta 7+$ liver-infiltrating lymphocytes (LIL) was increased relative to peripheral blood in PSC. In the only other study of the expression of MAdCAM-1 in human liver diseases, Hillan et al. detected MAdCAM-1 associated with portal tract inflammation in chronic hepatitis C and B as well as PBC and PSC [108].

In contrast to $\alpha 4\beta 7+$ LIL, CCR9+ LIL do appear to be specifically increased in PSC compared to PBC. Although the frequency of CCR9+ lymphocytes is not increased in peripheral blood, approximately 20% of LIL from PSC livers express CCR9 compared to <2% in normal livers or PBC [34]. This is in comparison to nearly 100% of lamina propria lymphocytes expressing CCR9+ in Crohn's disease. These CCR9+ LIL include CD8+ and CD4+ T cells, the former demonstrating a memory phenotype. Notably in apparent contradiction to their earlier work in which only 6% of CD3+ LIL expressed $\alpha 4\beta 7$ [36], Eksteen et al. reported that $\alpha 4\beta 7$ is co-expressed with CCR9. Nevertheless, the origin of these $\alpha 4\beta 7+$ CCR9+ lymphocytes being the intestine is supported by the recent finding that liver dendritic cells and stellate cells were unable to imprint these homing markers on CD8+ T cells [32].

The CCR9 ligand CCL25 also appears to be specifically upregulated in PSC liver. Furthermore, CCR9+ LIL preferentially migrate to CCL25 rather than to CXCL12 or CCL5 and are triggered by CCL25 to bind immobilized MAdCAM-1 via $\alpha 4\beta 7$.

Vascular adhesion protein-1 (VAP-1) is present on sinusoidal and vascular endothelium of most organs, including the liver with the greatest abundance found in high endothelial venules of peripheral lymph nodes. Neither bile ducts nor hepatocytes appear to express VAP-1 [109–111]. However, it does not belong to the selectin, integrin, or immunoglobulin super families [112]. In addition to adhesion of lymphocytes to peripheral lymph node high endothelial venules [113], it promotes shear-dependent lymphocyte adhesion to and transmigration across hepatic sinusoidal endothelium in vitro [109, 110, 114]. Further-

more, Lalor et al. reported that binding of benzylamine to VAP-1 in hepatic endothelial cells resulted in rapid activation of NF-κB. This led to expression of CXCL8, E-selectin, and ICAM-1 and upregulation of leukocyte adhesion [115]. Bonder et al. reported that Th1 cells use $\alpha 4\beta 1$ and Th2 cells use VAP-1 to adhere to sinusoidal epithelium. This was in contrast to previous theories that leukocyte recruitment in the sinusoids was mediated by physical trapping rather than adhesion [116]. The lymphocyte ligand of VAP-1 has not been identified and its role in the homing of lymphocytes to the liver in PSC has not been established.

Integrin $\alpha V\beta 6$ is expressed in large amounts on certain activated epithelia, mediating attachment to fibronectin and acting as a coreceptor for the activation of latent trans-forming growth factor (TGF)-$\beta 1$. In order to elucidate its role in liver fibrosis, Patsenker et al. studied $\alpha V\beta 6$ function in rats after bile duct ligation and in $Mdr2-/-$ mice. $\alpha V\beta 6$ was expressed in large amounts on proliferating bile duct epithelia in fibrosis. In addition, EMD527040, a $\alpha V\beta 6$ antagonist, decreased bile ductular proliferation and peri-biliary collagen deposition and downregulated fibrogenic genes while upregulating fibrolytic genes. It also reduced endogenous activation of TGF-$\beta 1$ [117]. In human liver, $\alpha V\beta 6$ is absent in normal liver but is expressed on activated bile duct epithelia and transitional hepatocytes. In chronic hepatitis C, integrin $\beta 6$ mRNA increases with stage of fibrosis. Thus, $\alpha V\beta 6$ does not appear to be a specific receptor targeting lymphocytes in PSC. Clearly, more research into the basic biology of lymphocyte homing to the liver and specificity of homing in PSC will expand our understanding of its pathogenesis tremendously.

Intestinal lymphocytes of PSC

If the liver inflammation of PSC is related to the aberrant homing of intestinal lymphocytes from IBD, perhaps the intestinal lymphocytes of the IBD associated with PSC differ phenotypically from IBD without PSC. A limited number of studies have examined intestinal lymphocytes from PSC patients and demonstrated that there are differences compared to IBD patients and healthy controls. PSC intestinal lymphocytes tend to have a lower frequency of IFN-γ and IL-4-producing cells and have a blunted proliferative response to IL-2 compared to IBD lymphocytes [118, 119]. Increased numbers of intestinal NK cells in PSC–IBD compared to IBD have also been documented [118].

Cytokines

Th1 inflammatory mediators are the predominant type in PSC. Bo et al. reported significantly higher levels of TNF-α and IL-1β and lower concentrations of IL-2, IL-10, and

IFN-γ in supernatants of LIL from PSC patients compared with PBC, AIH, and normal controls [99]. Anti-TNF-α antibodies restored the previously diminished proliferative response. Impaired cytolytic activity of NK and T cells was also observed, and this was partially restored after treatment with TNF-α antibody treatment. The authors concluded that it is unlikely that the injury in PSC is mediated by direct cytotoxic effects because of the inability to detect normal cytotoxic activity of the LIL. Consistent with this are the results of a recently completed pilot study of the anti-TNF-α monoclonal antibody infliximab which showed no improvement in liver biochemistry or histology compared to placebo.

Aoki et al. used RNA microarray analysis of peripheral blood mononuclear cells to identify immune-related genes and pathways that are differentially expressed in PSC. They discovered that genes within the IL-2 receptor beta, IL-6, and MAP kinase pathways were expressed differently in PSC patients compared to controls. They also noted that individual genes, TNF-α-induced protein 6 and membrane spanning 4-domains, subfamily A were upregulated in PSC while Mothers against decapentaplegic homolog 5 was downregulated. These findings are consistent with a systemic Th-1-mediated inflammatory response [120].

The effects of TNF-α and IFN-γ may also have specific effects on bile acid secretion in PSC. Spirli et al. demonstrated that TNF-α and INF-γ act synergistically to enhance nitric oxide production by increased nitric oxide synthase 2 (NOS2) gene and protein expression [121]. This in turn led to inhibition of cyclic-AMP dependent secretion by cholangiocytes. In contrast to PBC and chronic hepatitis C, PSC samples with advanced histologic stage had a significantly greater immunostaining of NOS2 in large and small bile ducts. The lack of increased staining in early stage PSC suggests that this change is in response to the underlying disease process, perhaps related to Th1-type responses to bouts of cholangitis.

Humoral immune responses

Autoantibodies are frequent in PSC but their pathologic significance remains unknown. Anti-neutrophil antibodies are often found in a perinuclear (p-ANCA) or atypical perinuclear pattern in approximately 80% of patients, but the diagnostic utility of this is somewhat limited because of the overlap with autoimmune hepatitis [122]. The atypical perinuclear pattern appears to be specific to PSC and AIH with staining of the nuclear periphery and multiple intra-nuclear foci. Both IgG and IgA classes of ANCA are prevalent in AIH and PSC; however, the atypical perinuclear staining pattern appears to be the predominant staining pattern for the IgG class in PSC. In contrast, the IgA class is found in an equal proportion of the atypical perinuclear pattern and the classical pattern. In patients with both IgG and IgA ANCA, IgA appears to be responsible for the classic p-ANCA staining while the IgG produces the atypical p-ANCA [123]. There is some preliminary evidence that the presence of p-ANCA may be able to be used as a prognostic indicator in PSC [124], although others have not been able to confirm this and the titer does not correlate with disease activity [123, 125].

The autoantigen to which both the classic and atypical p-ANCA in PSC react has yet to be identified. Orth et al. reported that catalase autoantibodies were present in 60% of their PSC cohort and alpha-enolase autoantibodies were present in 27% [126]. Catalase is an antioxidant that protects against cell damage from highly reactive oxygen-derived free radicals. Inhibition of catalase function might lead to increased oxidative stress in PSC. Bactericidal/permeability increasing (BPI) protein, an endotoxin-binding neutrophil leukocyte-granule protein that has antibacterial and anti-endotoxin properties, has also been suggested as a potential target of p-ANCA in PSC [127, 128]. Stoffel et al. reported that BPI-ANCA was present in 36% (13/36) of PSC patients compared to less than 10% of patients with ANCA-associated vasculitidies and disease controls [128]. They hypothesized that BPI-ANCA dampens the endotoxin-neutralizing ability of BPI leading to over-expression of bacterial antigenic stimuli. However, BPI-ANCA has also been found in cystic fibrosis, IBD, and vasculitis and is associated with higher inflammatory disease activity and greater organ damage [127].

More recently, it has been reported that atypical p-ANCA recognizes tubulin beta isoform 5 in human neutrophils and the bacterial cell division protein FtsZ which is present in almost all bacteria of the intestinal microflora [129, 130]. The authors suggest that this may reflect molecular mimicry in which autoantibodies triggered by a bacterial infection cross-react and inhibit normal immune cell functions [131]. Unfortunately, these results have only been published in preliminary form.

As mentioned above, there is evidence that BEC are targets of autoantibodies in PSC. Xu et al. reported that autoantibodies against surface antigens on BEC were found in a significantly greater percentage of PSC patients (63.3%) compared to PBC (37%) or AIH (16%) patients or normal controls (8%). Anti-BEC sera also caused BEC to increase expression of CD44 and production of IL-6 potentially leading to the recruitment of memory T cells via CD44 [132].

Concluding remarks

The etiopathogenesis of PSC remains enigmatic. Genetic susceptibility to the disease is clearly an important factor and as the specific genetic components are identified, better insights into the mechanisms of this disease will be

understood. Although progress has been made into the recruitment of lymphocytes from the gut to the liver, why chronic inflammation and fibrosis occurs only in a subset of IBD patients remains to be defined. In addition, an important question remains as to whether this same process holds true for the growing percentage of PSC patients without IBD. The lack of an adequate animal model and the relative infrequency of the disease remain major obstacles to progress and will only be overcome with close collaboration and focused research into the immunopathogenesis of PSC.

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