HEPATOLOGY

HEPATOLOGY, VOL. 72, NO. 3, 2020

The Role of the Intestine in the Pathogenesis of Primary Sclerosing Cholangitis: Evidence and Therapeutic Implications

Gregory Dean,[1] Stephen Hanauer,[2] and Josh Levitsky[2]

The pathogenesis of primary sclerosing cholangitis (PSC), a progressive biliary tract disease without approved medical therapy, is not well understood. The relationship between PSC and inflammatory bowel disease has inspired theories that intestinal factors may contribute to the development and progression of hepatobiliary fibrosis in PSC. There is evidence from both fecal and mucosa-associated microbial studies that patients with PSC harbor an abnormal enteric microbiome. These organisms are thought to produce toxic byproducts that stimulate immune-mediated damage of hepatocytes and the biliary tree. The link between these mechanisms may be related to altered intestinal permeability leading to migration of bacteria or associated toxins to the liver through the portal circulation. In support of these concepts, early trials have demonstrated improved biochemical parameters and symptoms of PSC with oral antibiotics, ostensibly through manipulation of the enteric microbiota. This article reviews the published literature for evidence as well as gaps in knowledge regarding these mechanisms by which intestinal aberrations might drive the development of PSC. We also identify areas of future research that are needed to link and verify these pathways to enhance diagnostic and therapeutic approaches. (Hepatology 2020;72:1127-1138).

Primary sclerosing cholangitis (PSC) is a chronic, inflammatory disease of the liver and bile ducts that leads to cholangitis, hepatobiliary fibrosis, and the potential need for liver transplantation. PSC also portends higher risk for both cholangiocarcinoma and colorectal cancers. No accepted medical therapy for PSC currently exists, in part because the etiology and pathogenesis of PSC are not well understood. In up to 80% of cases, PSC is associated with concomitant inflammatory bowel disease (IBD), more commonly ulcerative colitis (UC) than Crohn's disease (CD), although some now believe IBD associated with PSC to be a distinct entity.[1,2] The development of PSC does not typically correlate with IBD disease activity, and an extensive search for a genetic link has been unrevealing, except for a possible connection with autoimmunity.[3,4] The fact that PSC can recur after liver transplantation supports the idea that factors outside the liver itself may drive disease development.[5]

It has long been thought that abnormalities in the complex interplay between the gut and hepatobiliary system contribute to the pathogenesis of PSC. This is an alluring model, in part because it may explain the link between the enteric inflammation seen in IBD and the development of PSC. Figure 1 illustrates three key pathogenic mechanisms. First, there may be an altered population of gut microflora ("intestinal dysbiosis") in IBD and PSC that produces potentially toxic or immunostimulatory byproducts. Second,

Abbreviations: ALP, alkaline phosphatase; CD, Crohn's disease; HC, healthy controls; IBD, inflammatory bowel disease; PSC, primary sclerosing cholangitis; Th17, T-helper 17; UC, ulcerative colitis.

Received March 23, 2020; accepted April 29, 2020.
Financial Support: None.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.31311

Potential conflict of interest: Nothing to report.

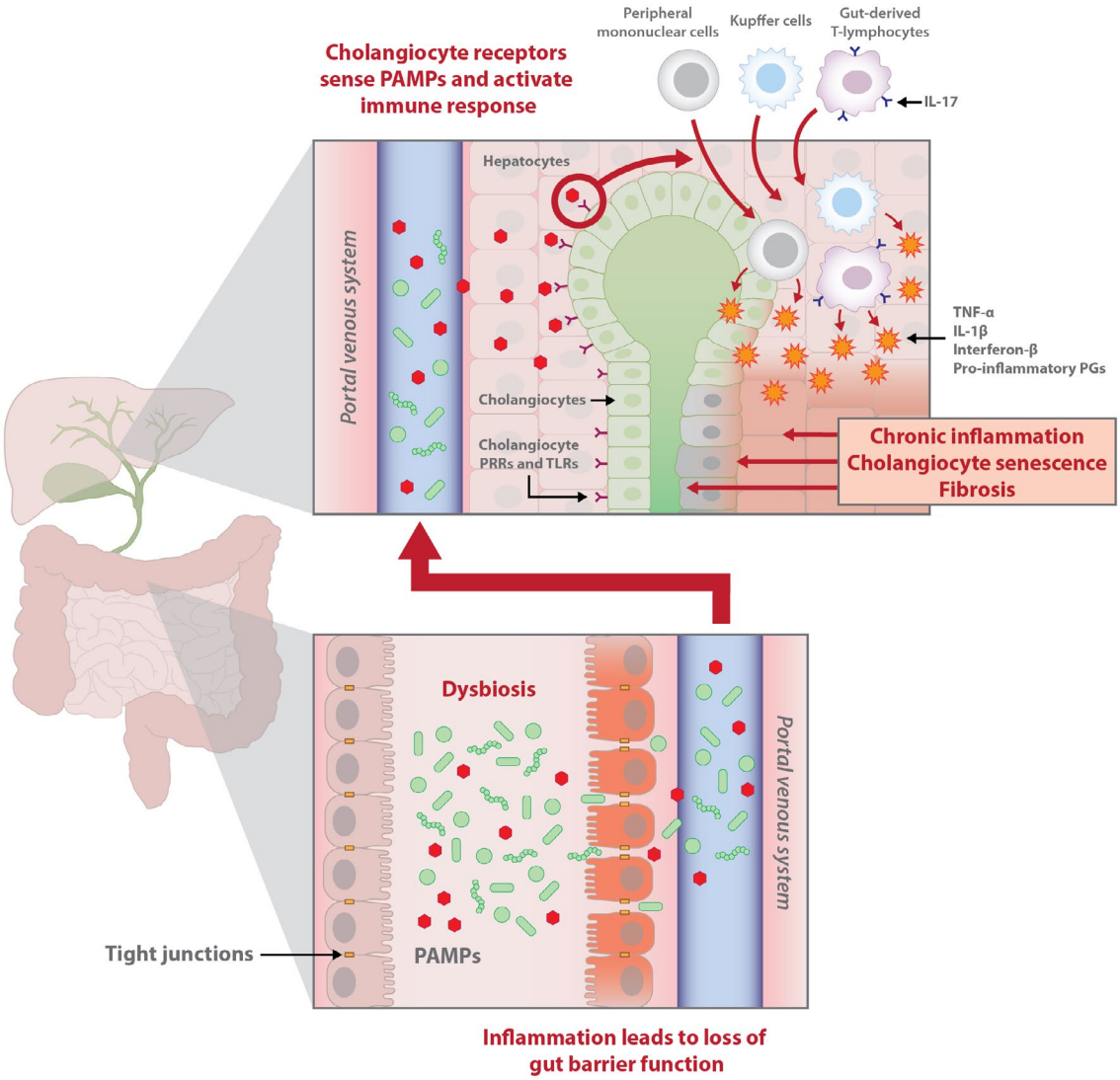

FIG. 1. PSC patients exhibit decreased enteric microbial diversity and altered species abundances (dysbiosis). These bacteria are thought to produce toxins, or PAMPs, which, in the setting of mucosal inflammation, translocate paracellularly into the portal venous system and travel to the liver. Here they are thought to stimulate an immune response, mediated by hepatic and peripheral lymphocytes as well as gut-derived T-lymphocytes which are activated by intestinal antigens. When chronic, this process leads to cholangiocyte senescence and fibrosis. Abbreviations: IL, interleukin; PAMP, pathogen-associated molecular protein; PG, prostaglandin; PRR, pattern recognition receptor; TLR, toll-like receptor; TNF-α, tumor necrosis factor-alpha.

ARTICLE INFORMATION:

From the [1]Department of Internal Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL; [2]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Josh Levitsky, M.D., M.S.
Division of Gastroenterology and Hepatology
Northwestern University Feinberg School of Medicine
676 N. St. Clair St., 19-045
Chicago, IL 60611
E-mail Josh.Levitsky@nm.org
Tel.: +1-312-926-9688

concomitant IBD may increase intestinal permeability through mucosal inflammation, allowing translocation of microbial toxins and bacteria to the hepatobiliary system. Finally, these bacteria or associated molecules may stimulate immune activation against hepatocytes and cholangiocytes, resulting in biliary injury, remodeling, and fibrosis. This article reviews the evidence for these three mechanisms by which microbiome alterations in the setting of altered intestinal permeability may lead to hepatobiliary inflammation and development of PSC. We also discuss potential therapeutic implications and future research directions related to the role of the intestine in PSC pathogenesis.

Intestinal Dysbiosis in IBD and PSC

The vital physiologic roles of commensal enteric microbiota in the conjugation of bile acids, production of vitamins, and facilitation of nutrient digestion have been well established. In general, high microbial diversity is considered a hallmark of a healthy enteric ecosystem as competition between commensal bacteria is thought to be related to population stability. The intestinal microbiome is now studied using high-throughput RNA sequencing to group species based on similarity of marker gene sequences within the highly conserved 16S ribosomal RNA subunit. Significant suppression of microbial diversity or derangement in bacterial species prevalence is referred to as "dysbiosis" and has been reported in a wide variety of conditions.[6] In animals, small intestinal bacteria overgrowth can lead to hepatocyte necrosis and small-duct biliary inflammation resembling early-stage PSC, which can be attenuated by antibiotic therapy.[7,8]

Decreased microbial diversity and species-specific alterations have been reported in PSC, with and without IBD (Tables 1 and 2). Most studies use fecal samples due to the ease of collection and abundance of bacteria. Some investigators believe that fecal samples may not be an accurate representation of mucosa-associated bacterial populations; thus, others have studied bacteria derived from colonoscopic mucosal biopsies. These results, however, may be limited by bowel preparations for endoscopy that may alter microbial populations and have less power to detect changes given the relative paucity of bacteria that can be grown from biopsied tissue. An advantage of mucosal studies is that the degree of inflammation (i.e., IBD activity) can be precisely characterized on biopsy. Although some microbiota studies require that patients with PSC-IBD be in remission, many studies have included patients with varying degrees of active inflammation, which is likely a confounder given that dysbiosis has been widely reported in IBD alone.[9]

Compared to healthy controls (HC), patients with PSC have significant suppression of global diversity indices (intraindividual or α-diversity) as well as differences in population composition and species abundance between sampling units (β-diversity).[10-17] In the largest study showing these differences, Rühlemann et al. compared 127 patients with PSC from Norway and Germany to 118 patients with UC alone and 133 HC.[15] Interestingly, the majority of core microbiota was shared between German and Norwegian patients, although there were small differences in both α-diversity and β-diversity between the two. This is an important consideration as geographic differences in microbiota may significantly contribute to microflora variability. Mucosal studies have similarly found reduced α-diversity and β-diversity in patients with PSC compared to HC, although these results have not been consistent across studies.[18-21]

Fecal studies also provide evidence that patients with PSC harbor genus-specific and species-specific changes compared to HC. Multiple studies have found significant increases in the abundance of *Veillonella*, *Enterococcus*, and *Streptococcus*. *Veillonella*, an amine-oxidase expressing organism, could play a role in aberrant gut lymphocyte tracking to the liver, while *Enterococcus* is a vancomycin-sensitive organism that has been found to contribute to intestinal barrier disruption and inflammation through a metalloprotease that cleaves epithelial cadherins.[22] Studies have also found increases in the class Gammaproteobacteria, which comprises Enterobacteriaceae and common gastrointestinal pathogens such as *Klebsiella* and *Proteus*.[14,15] Others have analyzed fungal dysbiosis, finding several species-specific differences, including decreased *Saccharomyces cerevisiae*, which is interesting as PSC has been associated with anti–*S. cerevisiae* antibodies.[13,23] Meanwhile, evidence for species-specific changes in PSC obtained from mucosal biopsies has been less convincing than data from fecal samples, although two studies found major shifts in

TABLE 1. Studies of Fecal Microbiota in PSC

	Kummen et al.[12]	Sabino et al.[16]	Iwasawa et al.[11]	Bajer et al.[10]
Year	2017	2016	2017	2017
Patients with PSC	85	66	13	43
Age (mean)	49	49	15	40
PSC-IBD (%)	55 (65)	48 (73)	13 (100)	32 (74)
PSC-UC (%)	44 (52)	27 (41)	6 (46)	
PSC-CD (%)	11 (13)	21 (32)		
PSC duration (mean, years)	9.1			
IBD controls	36	43	15	32
Age (mean)	40	50	13	40
HC	263	66	23	31
Age (mean)	46	52	12	44
α-Diversity				
PSC vs. IBD	←→	↑	↑	
PSC vs. HC	↓	↓	↓	↓
β-Diversity				
PSC vs. IBD	Different	Different	Different	
PSC vs. HC	Different	Different	Different	Different
PSC vs. IBD-PSC	Similar	Similar		Different
IBD activity IBD alone	Quiescent	Mean IBD activity score 2.5; median CRP 2.15	50% in remission, 31% mild, 19% moderate/severe	62.5% mild/remission, 9.4% moderate, 9% severe
IBD activity PSC-IBD	Quiescent	Mean IBD activity score 0.5; median CRP 2.15	70% in remission, 30% mild, none moderate/severe	75% mild/remission, 12.5% moderate, 12.5% severe
Taxa increased PSC vs. HC	*Viellonella*	*Veillonella, Streptococcus, Enterococcus, Lactobacillus, Fusobacterium*	*Veillonella, Streptococcus, Enterococcus*	*Veillonella, Rothia, Streptococcus, Enterococcus*
Taxa decreased PSC vs HC	*Coproccoccus, Phascolarctobacterium,* Lachnospiraceae Christensenellaceae			*Coprococcus*

	Torres et al.[17]	Lemoinne et al.[13]	Rühlemann et al.[15]	Nakamoto et al.[14]
Year	2018	2020	2019	2019
Patients with PSC	15	49	137	18
Age (mean)	42	41	47	33
PSC-IBD (%)	15 (100%)	27 (55)	75 (55%)	18 (100%)
PSC-UC (%)	11 (73%)	12 (24)		18 (100%)
PSC-CD (%)	4 (27%)	11 (22)		0 (0%)
PSC duration (mean, years)	7.8	6	8,2	
IBD controls	15	33	118	16
Age (mean)	45	36	43	39
HC		30	133	10
Age (mean)		31	47	32
α-Diversity				
PSC vs. IBD	←→		←→ (Norwegian), ↑ (German)	←→
PSC vs. HC		↓	↓ (Norwegian), ←→ (German)	↓
β-Diversity				
PSC vs. IBD	Different	Different	Different	
PSC vs. HC		Different	Different	

TABLE 1. *Continued*

	Torres et al.[17]	Lemoinne et al.[13]	Rühlemann et al.[15]	Nakamoto et al.[14]
PSC vs. IBD-PSC		Different	Similar	
IBD activity IBD alone	87% remission/mild, 13% moderate/severe	Quiescent	Median fecal calprotectin 43.3	Mayo 0:2, 1:3, 2:6, 3:5; mean CRP 0.28
IBD activity PSC-IBD	64% remission/mild, 36% moderate/severe	Quiescent	Median fecal calprotectin 29.4	Mayo 0:1, 1:8, 2:1, 3:0; mean CRP 0.20
Taxa increased PSC vs. HC	Increased vs. IBD: *Ruminococcus, Fusobacterium*	*Exophiala* (fungal), *Veillonella*, Sphingomonadaceae, Alphaproteobacteria, Rhizobiales	*Veillonella, Streptococcus, Enterococcus, Lactobacillus, Parabacterioides*, Gammaproteobacteria	*Enterococcus gallinarum, Klebsiella pneumoniae, Proteus mirabilis*
Taxa decreased PSC vs. HC	*Blautia, Roseburia, Veillonella, Dorea*	*Saccharomyces cereviseae* (fungal) *Ruminococcus, Ruminiclostridium, Faecalibacterium, Lachnoclostridium, Blautia*	*Coprococcus*	

Abbreviation: CRP, C-reactive protein.

TABLE 2. Studies of Mucosal Microbiota in PSC

	Rossen et al.[21]	Torres et al.[19]	Kevans	Quraishi et al.[18]
Year	2015	2016	2016	2017
Patients with PSC	12	20	31	11
Age (mean)	29.5	47	43	
PSC-IBD (%)	12 (100)	19 (95)	31 (100)	11 (100)
PSC-UC (%)	8 (67)	13 (65)	31 (100)	
PSC-CD (%)	4 (33)	6 (30)	0	
Disease duration (mean, years)	2	4	1.3	
IBD controls	11	15	30	10
Age (mean)	50	48		
HC	9	9		9
Age (mean)	65	65		
α-Diversity				
PSC vs. IBD		←→	←→	
PSC vs. HC	↓	←→		
β-Diversity				
PSC vs. IBD	No difference	No difference	No difference	Different
PSC vs. HC	No difference			Different
PSC vs. IBD-PSC				
IBD activity IBD alone	18% with endoscopic disease activity	73% quiescent, 27% mild/moderate	Mayo endoscopic score ≤ 1	
IBD activity PSC-IBD	21% with endoscopic disease activity	55% quiescent, 45% mild/moderate	Mayo endoscopic score ≤ 1	
Taxa increased in PSC vs. HC		*Blautia, Ruminococcus*		*Escherichia, Megasphaera,*
Taxa decreased in PSC vs. HC	Clostridiales			*Prevotella, Roseburia, Bacteroides*

Clostridiales populations.[19,21] One notable finding in two mucosal studies was that bacterial populations did not differ significantly at different sites in the colon.[18,19]

Some work suggests that the severity of liver disease may correlate with the severity of dysbiosis. Sabino et al. found that patients with cirrhosis or needing liver transplant had more extreme

deviations in several genera from HC.[16] It is possible that hepatic dysfunction, including changes in bile acid metabolism or portal hypertension, could contribute to dysbiosis. Investigation of dysbiosis in a range of other liver diseases has revealed similar associations, raising the question of how specific these changes are to PSC.[6,24] Notably, none of these studies included a non-PSC cholestatic control group.

It is also useful to compare patients with PSC with and without IBD to patients with IBD alone, especially when considering the confounding effect of intestinal inflammation on the enteric microbiome. Suppression of microbial diversity has been well established in IBD alone.[9] Fecal and mucosal studies have consistently found differences in β-diversity between PSC (with or without IBD) and IBD alone, indicating that patients with PSC harbor distinct enteric microbial populations. Some have actually found that there is more extreme suppression of global diversity in IBD alone than PSC, although patients with IBD in these cohorts tended to have higher intestinal inflammatory activity than patients with PSC-IBD. This raises the likelihood that active inflammation in IBD contributes to dysbiosis.[11,15,16]

Finally, studies disagree on whether patients with PSC-IBD have different microbial profiles from patients with PSC alone. Rühlemann et al. found that there was no significant difference in β-diversity between PSC alone and PSC-IBD, indicating that PSC-specific alterations may occur independently of IBD.[15] Similarly, Kummen et al. found that the global microbial signature was comparable in PSC with and without IBD.[12] Notably, all patients in this study were reported to be in IBD remission. In contrast, Bajer et al. found that patients with PSC-IBD had more extreme decreases in diversity than patients with PSC alone, although this study included a significant portion of patients in both groups who had active inflammation.[10] The contrast between these studies again highlights the importance of factoring in IBD activity in these analyses.

In summary, there is evidence that patients with PSC harbor an enteric microbiome characterized by globally reduced diversity and different species profiles from patients with IBD and HC. This is especially notable regarding the prevalence of *Veillonella* and *Enterococcus*, two organisms which may have a mechanistic basis for pathogenic activity. It is unclear if microbial populations differ between PSC-IBD and PSC alone, and it may be dependent on IBD activity. Evidence from mucosal biopsy studies generally echoes the findings of fecal studies with respect to reduced diversity and some PSC-specific species alterations. Despite this evidence, we cannot yet determine whether these alterations reflect a cause or effect of PSC or the specificity of these changes for PSC. Efforts are needed to determine the time course of developing dysbiosis at different points of disease development and progression, as well as to account for IBD activity and environmental variability.

Intestinal Permeability and Bacterial Translocation

An intact gut epithelium provides complex antimicrobial mucous layers and intercellular tight junctions that serve as a first line of defense against invasion by pathogens and commensal bacteria. In the setting of mucosal injury, bacteria and toxins can translocate paracellularly through disrupted tight junctions into the portal circulation.[25] Both small and large bowel permeability can be studied using functional assays that compare the absorption of poorly or nonabsorbed oligosaccharides; higher urinary elimination of larger molecules represents increased intestinal permeability.[26] Similar to intestinal dysbiosis, increased intestinal permeability has been extensively reported in many disease states.[27] Most of these studies have evaluated small bowel permeability as assessment of large bowel permeability requires a longer duration of urine collection (up to 24 hours, to allow for transit time to the colon) as well as use of alternative probes that are not degraded by colonic bacteria.[28] There is strong evidence for increased small intestinal permeability in IBD, particularly CD, as well as first-degree relatives of these patients.[29] There is also some evidence of increased small and large bowel permeability in UC.[30-34] Studies in both UC and CD have reported increased permeability in the setting of quiescent disease; however, others have also found the degree of permeability not only to correlate with endoscopic disease activity but to attenuate with treatment.[29]

In PSC, we identified only one Swedish study that failed to show differences in small bowel permeability in PSC with or without IBD compared to HC.[35]

However, this study was small in size and did not assess for colonic permeability, which requires the extended methodology described above. Thus, studies of larger size and comprehensive small and large bowel permeability assessments in PSC with and without IBD are needed to establish the key initial question of whether intestinal permeability is truly altered in PSC. The fact that UC tends to be more clinically quiescent in patients with concomitant PSC argues against the theory that intestinal inflammation causing increased colonic permeability contributes to PSC development.[1,2,36] However, evidence showing that patients who underwent colectomy prior to or at the time of liver transplantation may have less risk of developing recurrent PSC in the transplanted liver supports this idea.[37] Although severity of IBD activity and PSC disease course do not appear to be connected in epidemiological studies, research has yet to determine if treatment of IBD, quiescent or not, alters the natural history of PSC.[3]

A more indirect way to test for intestinal permeability is to assess for translocation of bacteria or bacterial antigens across the gut barrier into the portal circulation. This is difficult to study in humans due to the need for invasive portal venous sampling. Several studies have shown that a high percentage of patients with PSC, especially those with significant biliary stenoses, have a higher prevalence of enteric bacteria present in bile duct samples.[38-40] Given the challenges intrinsic to studying portal or biliary tracts, investigators have also focused on peripheral blood microbial byproducts, such as lipopolysaccharide, a gram-negative endotoxin, and lipoteichoic acid, a gram-positive cell wall component. Multiple studies have correlated peripheral endotoxemia with both acute and chronic liver disease.[41-44] In patients with alcoholic liver disease, Parlesak et al. found increased small and large intestinal permeability to polyethylene glycol of molecular weight similar to endotoxin, as well as peripheral endotoxemia. Notably, patients with more advanced liver disease did not have more extreme changes in permeability or endotoxemia than those with normal transaminases and liver function tests, indicating that these changes may be related to toxic exposures in the gut rather than hepatic dysfunction.[44] In an elegant translational experiment, Nakamoto et al. transplanted fecal microbiota from patients with PSC-UC, patients with UC, and healthy patients into germ-free mice. PSC-UC mice showed increased levels of serum endotoxin, and the same bacteria that were altered in the feces of patients with PSC-UC (Table 1) were found in the mesenteric lymph nodes of transplanted mice. One strain of *Klebsiella* directly invaded the intestinal mucosa of transplanted mice, leading to increased gut permeability and endotoxemia.[14] The fact that these changes occurred in the absence of liver disease highlights the possible link between dysbiosis, intestinal permeability, and bacterial translocation.

Hepatobiliary Inflammation Due to Intestinal Alterations

Proof-of-concept for the hypothesis that immune-mediated hepatobiliary injury might result from intestinal factors comes from animal models showing that intentional overgrowth of intestinal bacteria or fecal administration of bacterial byproducts can lead to hepatobiliary inflammation resembling PSC.[8,45,46] In fact, the liver is continuously exposed to a wide variety of potential enteric antigens under physiologic conditions and thus must have a finely tuned balance between protective immune responses and tolerance in order to avoid tissue injury. Intestinal flora can generate pathogen-associated molecular proteins that activate proinflammatory cascades, propagated by both hepatic macrophages and cholangiocytes.[3,47] In PSC, the complex immune response orchestrated by cholangiocytes has been the subject of intense investigation. Cytokine mediators from these cells, produced in response to microbial components, have been shown to lead to hepatic and peripheral inflammatory cell chemotaxis to the biliary tree, myofibroblast proliferation and differentiation, and cholangiocyte senescence and apoptosis.[48-50] When chronically activated, the result of this cascade is remodeling, fibrosis, and eventual obliteration of the bile ducts.

Cholangiocytes from explanted livers of patients with PSC display inappropriate immune signaling in response to endotoxins compared to non-PSC explants.[48] There is other evidence that patients with PSC exhibit inappropriate immune responses to intestinal factors as well. The aforementioned

Nakamoto study found up-regulation of inflammatory and fibrosis gene expression, as well as T-helper 17 (Th17) cell priming in the livers and colon of PSC-UC mice after fecal transplantation from patients with PSC.[14] Katt et al. used bacteria isolated from biliary fluid of patients with PSC to stimulate peripheral blood mononuclear cells, finding that patients with PSC exhibited a greater Th1 and Th17 response than HC or patients with primary biliary cholangitis and that patients with PSC-IBD did not differ in this response from patients with PSC alone.[51] These findings indicate that the Th17-cell response may play a critical role in promoting fibrosis in patients with PSC; the importance of these cells in mucosal immunity and the ability of interleukin-17 to promote hepatic inflammation and fibrosis has been established.[52,53]

Enterohepatic bile acid circulation is also a key regulator of the "gut–liver axis" and is thought to play an important role in the immune response. Primary bile acids synthesized in the liver are metabolized by gut bacteria and then recycled back to the liver through enterocyte uptake, mediated by the farsenoid X receptor and Takeda G-protein-coupled receptor 5. Recent work shows that binding of bile acids to these receptors induces antimicrobial peptide production, enhances fibroblast growth factor production, and modulates metabolism.[54-56] Intestinal dysbiosis has been shown to alter the balance of primary and secondary bile acids, which could modulate these complex signaling pathways.[57]

Another theory holds that gut-derived T lymphocytes, activated in response to intestinal antigens or by episodes of intestinal inflammation, home to the liver and initiate immune-mediated damage. This process may be facilitated in PSC by abnormal hepatic expression of endothelial cell adhesion molecules such as mucosal vascular adhesion cell adhesion molecule 1 (MAd-CAM-1), which is typically limited to the gut, and vascular adhesion protein-1 (VAP-1), a protein that facilitates leukocyte transmigration across vascular endothelia.[58] Similarly, the work of Adams and Afford suggests that a network of chemokine receptors that are normally restricted to the gut are aberrantly expressed in the liver, leading to the recruitment of intestinal lymphocytes through enterohepatic circulation.[59] Other studies have found phenotypic differences in both circulating as well as colonic lymphocytes of patients with PSC compared to patients with UC or HC.[60-62]

The degree to which differences in lymphocyte profiles and immune signaling can be attributed to intestinal factors is unclear. There is also likely an underlying genetic basis; gene studies have identified 23 or more susceptibility loci in PSC, with the strongest associations being with the human leukocyte antigen (HLA) complex. The genetic overlap with IBD is not as strong as initially suspected, again suggesting that PSC-IBD is a unique entity.[4,62] Further, siblings of patients with PSC do have an increased risk of developing PSC similar to other autoimmune conditions. However, the largest genome-wide association studies suggest that genetic factors explain only a small proportion of overall PSC liability.[4,63] It is likely that PSC develops in genetically susceptible individuals in response to immune triggers, including intestinal antigens. However, specific antigenic stimuli, enteric or otherwise, have not yet been correlated with these HLA loci.[4]

Targeting Intestinal Alterations for PSC Treatment Strategies

There are no approved medical therapies for PSC. Immunosuppressive agents, anti-inflammatory drugs, and bile acid therapy (ursodeoxycholic acid [UDCA]) have not led to improvement in disease course. Therapies aimed at manipulation of the enteric flora, intestinal permeability, and inflammatory response to microbes could potentially delay PSC progression or even the onset of disease. Surrogate endpoints are now being used to define clinical response as several of the major clinical endpoints (death, liver transplantation) take years to occur in trials. Studies have found that alkaline phosphatase (ALP) reduction to <1.5 times the upper limit of normal is associated with increased time to death, liver transplantation, and development of cholangiocarcinoma.[64,65]

In small clinical trials, administration of oral antibiotics has been shown to lower ALP and liver enzymes and, in some cases, to alleviate symptoms of PSC. Tabibian et al. achieved a primary endpoint of

> 40% reduction in ALP with administration of both low-dose and high-dose vancomycin as well as high-dose metronidazole in 35 patients with PSC. Further, the Mayo PSC risk score, a commonly used clinical tool designed to predict mortality in PSC, decreased significantly in both low-dose groups, and pruritus decreased in the high-dose metronidazole group.[66] Davies et al. found significant improvement in liver enzymes and clinical symptoms in response to oral vancomycin in nearly all 14 pediatric patients with PSC; further, there was recrudescence of symptoms after discontinuation of therapy.[67] Other small studies have found improvement of symptoms in patients with PSC on oral antibiotics as well.[68]

The mechanism of action of antibiotic therapy has not been confirmed. Although it may involve suppression of pathogenic enteric bacteria, pre-therapy and post-therapy microbial analyses have not yet been performed. Independent from its antibiotic properties, vancomycin may have immunomodulatory effects on the tumor necrosis factor-alpha and transforming growth factor-beta (TGF-β) pathways.[69,70] Abarbanel et al. reported that pediatric patients with PSC had increased levels of regulatory T cells and TGF-β after vancomycin therapy, parallel to improvement in liver biochemistry, biliary imaging, inflammation in liver and intestinal biopsies, and IBD symptoms.[71] Whether immunomodulation was a driver of disease improvement in this study or an effect of antibiosis is uncertain.

Finally, alternative therapies such as fecal transplant and probiotics have also been explored. Allegretti et al. treated 6 quiescent patients with PSC-IBD (mostly UC) with fecal transplant and found increased microbial diversity in fecal samples following treatment as well as a reduction in ALP in 3 of the 6.[72] Vleggaar et al. treated 14 patients with PSC with probiotics but did not find any improvement in lab parameters.[73] More studies with larger sample sizes are needed to assess the efficacy of these treatments.

Taken together, the intentional alteration of intestinal microflora appears to be a promising therapeutic target in preliminary studies. Skepticism over biochemical improvement alone is understandable in PSC, given that UDCA was shown to lead to biochemical improvement but not to slow progression of disease and may be harmful in high doses.[74] Studies of longer duration demonstrating symptomatic benefit and improvement in other meaningful endpoints are needed to understand the true benefit of antibiotic therapy as well as determine their mechanisms of action.

Summary and Future Directions

We have highlighted several lines of evidence that intestinal alterations such as dysbiosis, altered permeability, and dysregulated enterohepatic immune signaling may contribute to the pathogenesis of PSC. Modification of the intestinal microbiome and immune activation may improve biochemical endpoints and clinical symptoms of PSC. However, this review has identified several key questions that still need to be answered to link these pathways and validate treatment strategies.

First, we cannot yet determine if PSC is the cause or an effect of altered intestinal permeability. While increased intestinal permeability is often cited as a critical link in the pathogenic pathway, this has yet to be demonstrated in humans with PSC. Thus, in clinical studies, patients with mild, early-stage PSC with and without quiescent IBD need to have simultaneous assessments of the fecal and mucosal microbiome, small and large bowel permeability, and systemic inflammatory analyses in order to have a true baseline. From here, we can better compare to patients with later-stage disease and analyze PSC versus PSC with IBD as well as type (UC versus CD) and location of IBD.

Second, IBD activity has thus far been inadequately controlled for in studies. Studies of PSC should use quiescent patients with IBD but also separately explore the effect of IBD activity in PSC pathogenesis in order to more adequately elucidate the relative contribution of gut inflammation and microbial changes to the pathogenesis of PSC. These studies may provide further evidence of our hypothesis that intestinal alterations are key to the development of PSC. Alternatively, there could be separate nonintestinal pathways in patients with PSC alone that need deeper exploration. Some intestinal alterations may occur in the absence of observable inflammation, as with first-degree relatives of patients with IBD who exhibit similar enteric dysbiosis.[29] In addition, with intermittent endoscopic procedures alongside random biopsy sampling, it is

possible that quiescent, undiagnosed IBD is missed in the PSC population. Further study of this phenomenon may lead to earlier and/or more aggressive treatments for patients with PSC and IBD. The ultimate goal is to determine if therapies targeting these pathways modulate the disease course of PSC and reduce the development of serious, life-threatening complications.

Acknowledgment: For design of Figure 1, we thank Peter Tran, B.S., graduate student, Department of Chemical and Biological Engineering, Northwestern University.

Author Contributions: All three authors meet the ICMJE definition of authorship. By CRediT taxonomy: G.D., conceptualization, investigation, methodology, validation, writing - original draft, writing - review and editing; S.H., conceptualization, investigation, methodology, supervision, validation, writing - review and editing; J.L., conceptualization, investigation, methodology, supervision, validation, writing - review and editing.

REFERENCES

1) Weismüller TJ, Trivedi PJ, Bergquist A, Imam M, Lenzen H, Ponsioen CY, et al. Patient age, sex, and inflammatory bowel disease phenotype associate with course of primary sclerosing cholangitis. Gastroenterology 2017;152:1975-1984.e78.
2) Loftus EV, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, et al. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. Gut 2005;54:91-96.
3) Aron JH, Bowlus CL. The immunobiology of primary sclerosing cholangitis. Semin Immunopathol 2009;31:383-397.
4) Jiang X, Karlsen TH. Genetics of primary sclerosing cholangitis and pathophysiological implications. Nat Rev Gastroenterol Hepatol 2017;14:279-295.
5) Kugelmas M, Spiegelman P, Osgood MJ, Young DA, Trotter JF, Steinberg T, et al. Different immunosuppressive regimens and recurrence of primary sclerosing cholangitis after liver transplantation. Liver Transpl 2003;9:727-732.
6) Biedermann L, Rogler G. The intestinal microbiota: its role in health and disease. Eur J Pediatr 2015;174:151-167.
7) Lichtman SN, Keku J, Clark RL, Schwab JH, Sartor RB. Biliary tract disease in rats with experimental small bowel bacterial overgrowth. Hepatology 1991;13:766-772.
8) Lichtman SN, Keku J, Schwab JH, Sartor RB. Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. Gastroenterology 1991;100:513-519.
9) Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology 2014;146:1489-1499.
10) Bajer L, Kverka M, Kostovcik M, Macinga P, Dvorak J, Stehlikova Z, et al. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. World J Gastroenterol 2017;23:4548-4558.
11) Iwasawa K, Suda W, Tsunoda T, Oikawa-Kawamoto M, Umetsu S, Inui A, et al. Characterisation of the faecal microbiota in Japanese patients with paediatric-onset primary sclerosing cholangitis. Gut 2017;66:1344-1346.
12) Kummen M, Holm K, Anmarkrud JA, Nygård S, Vesterhus M, Høivik ML, et al. The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls. Gut 2017;66:611-619.
13) Lemoinne S, Kemgang A, Ben Belkacem K, Straube M, Jegou S, Corpechot C, et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. Gut 2020;69:92-102.
14) Nakamoto N, Sasaki N, Aoki R, Miyamoto K, Suda W, Teratani T, et al. Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune response in primary sclerosing cholangitis. Nat Microbiol 2019;4:492-503.
15) Rühlemann M, Liwinski T, Heinsen FA, Bang C, Zenouzi R, Kummen M, et al. Consistent alterations in faecal microbiomes of patients with primary sclerosing cholangitis independent of associated colitis. Aliment Pharmacol Ther 2019;50:580-589.
16) Sabino J, Vieira-Silva S, Machiels K, Joossens M, Falony G, Ballet V, et al. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. Gut 2016;65:1681-1689.
17) Torres J, Palmela C, Brito H, Bao X, Ruiqi H, Moura-Santos P, et al. The gut microbiota, bile acids and their correlation in primary sclerosing cholangitis associated with inflammatory bowel disease. United European Gastroenterol J 2018;6:112-122.
18) Quraishi MN, Sergeant M, Kay G, Iqbal T, Chan J, Constantinidou C, et al. The gut-adherent microbiota of PSC-IBD is distinct to that of IBD. Gut 2017;66:386-388.
19) Torres J, Bao X, Goel A, Colombel JF, Pekow J, Jabri B, et al. The features of mucosa-associated microbiota in primary sclerosing cholangitis. Aliment Pharmacol Ther 2016;43:790-801.
20) Kevans D, Tyler AD, Holm K, Jørgensen KK, Vatn MH, Karlsen TH, et al. Characterization of intestinal microbiota in ulcerative colitis patients with and without primary sclerosing cholangitis. J Crohns Colitis 2016;10:330-337.
21) Rossen NG, Fuentes S, Boonstra K, D'Haens GR, Heilig HG, Zoetendal EG, et al. The mucosa-associated microbiota of PSC patients is characterized by low diversity and low abundance of uncultured Clostridiales II. J Crohns Colitis 2015;9:342-348.
22) Steck N, Hoffmann M, Sava IG, Kim SC, Hahne H, Tonkonogy SL, et al. *Enterococcus faecalis* metalloprotease compromises epithelial barrier and contributes to intestinal inflammation. Gastroenterology 2011;141:959-971.
23) Muratori P, Muratori L, Guidi M, Maccariello S, Pappas G, Ferrari R, et al. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) and autoimmune liver diseases. Clin Exp Immunol 2003;132:473-476.
24) Anand G, Zarrinpar A, Loomba R. Targeting dysbiosis for the treatment of liver disease. Semin Liver Dis 2016;36:37-47.
25) Berg RD. Bacterial translocation from the gastrointestinal tract. Adv Exp Med Biol 1999;473:11-30.
26) Arrieta MC, Bistritz L, Meddings JB. Alterations in intestinal permeability. Gut 2006;55:1512-1520.
27) Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, et al. Intestinal permeability—a new target for disease prevention and therapy. BMC Gastroenterol 2014;14:189.
28) Meddings JB, Gibbons I. Discrimination of site-specific alterations in gastrointestinal permeability in the rat. Gastroenterology 1998;114:83-92.
29) Teshima CW, Dieleman LA, Meddings JB. Abnormal intestinal permeability in Crohn's disease pathogenesis. Ann N Y Acad Sci 2012;1258:159-165.

30) Rao A, Camilleri M, Burton DD, Eckert DJ, Pardi DS, Singh RJ, et al. Comparison of small bowel and colonic mucosal permeability in ulcerative/microscopic colitis, irritable bowel syndrome-diarrhea, and healthy controls by urinary saccharide excretion measurements. Gastroenterology 2011;140(Suppl. 1): S707-S708.
31) Büning C, Geissler N, Prager M, Sturm A, Baumgart DC, Büttner J, et al. Increased small intestinal permeability in ulcerative colitis: rather genetic than environmental and a risk factor for extensive disease? Inflamm Bowel Dis 2012;18:1932-1939.
32) Den Hond E, Hiele M, Evenepoel P, Peeters M, Ghoos Y, Rutgeerts P. In vivo butyrate metabolism and colonic permeability in extensive ulcerative colitis. Gastroenterology 1998;115:584-590.
33) Arslan G, Atasever T, Cindoruk M, Yildirim IS. (51)CrEDTA colonic permeability and therapy response in patients with ulcerative colitis. Nucl Med Commun 2001;22:997-1001.
34) Almer S, Franzén L, Olaison G, Smedh K, Ström M. Increased absorption of polyethylene glycol 600 deposited in the colon in active ulcerative colitis. Gut 1993;34:509-513.
35) Björnsson E, Cederborg A, Akvist A, Simren M, Stotzer PO, Bjarnason I. Intestinal permeability and bacterial growth of the small bowel in patients with primary sclerosing cholangitis. Scand J Gastroenterol 2005;40:1090-1094.
36) Van Der Have M, Oldenburg B. Is ulcerative colitis associated with primary sclerosing cholangitis an undertreated condition? Inflamm Bowel Dis 2020;26:780-781.
37) Alabraba E, Nightingale P, Gunson B, Hubscher S, Olliff S, Mirza D, et al. A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts. Liver Transpl 2009;15:330-340.
38) Pohl J, Ring A, Stremmel W, Stiehl A. The role of dominant stenoses in bacterial infections of bile ducts in primary sclerosing cholangitis. Eur J Gastroenterol Hepatol 2006;18:69-74.
39) Olsson R, Björnsson E, Bäckman L, Friman S, Höckerstedt K, Kaijser B, et al. Bile duct bacterial isolates in primary sclerosing cholangitis: a study of explanted livers. J Hepatol 1998;28:426-432.
40) **Liwinski T**, **Zenouzi R**, John C, Ehlken H, Rühlemann MC, Bang C, et al. Alterations of the bile microbiome in primary sclerosing cholangitis. Gut 2020;69:665-672.
41) Fukui H, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J Hepatol 1991;12:162-169.
42) Raparelli V, Basili S, Carnevale R, Napoleone L, Del Ben M, Nocella C, et al. Low-grade endotoxemia and platelet activation in cirrhosis. Hepatology 2017;65:571-581.
43) Wang J, Chen M, Sun G, et al. Small bowel bacterial overgrowth and endotoxemia in cirrhosis. [in Chinese] Chin J Intern Med 2002;41:459-461.
44) Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J Hepatol 2000;32:742-747.
45) Hobson CH, Butt TJ, Ferry DM, Hunter J, Chadwick VS, Broom MF. Enterohepatic circulation of bacterial chemotactic peptide in rats with experimental colitis. Gastroenterology 1988;94:1006-1013.
46) Yamada S, Ishii M, Liang LS, Yamamoto T, Toyota T. Small duct cholangitis induced by N-formyl L-methionine L-leucine L-tyrosine in rats. J Gastroenterol 1994;29:631-636.
47) Giordano DM, Pinto C, Maroni L, Benedetti A, Marzioni M. Inflammation and the gut-liver axis in the pathophysiology of cholangiopathies. Int J Mol Sci 2018;19:3003.
48) Mueller T, Beutler C, Picó AH, Shibolet O, Pratt DS, Pascher A, et al. Enhanced innate immune responsiveness and intolerance to intestinal endotoxins in human biliary epithelial cells contributes to chronic cholangitis. Liver Int 2011;31:1574-1588.
49) Syal G, Fausther M, Dranoff JA. Advances in cholangiocyte immunobiology. Am J Physiol Gastrointest Liver Physiol 2012;303:G1077-G1086.
50) Tabibian JH, O'Hara SP, Splinter PL, Trussoni CE, LaRusso NF. Cholangiocyte senescence by way of N-ras activation is a characteristic of primary sclerosing cholangitis. Hepatology 2014;59:2263-2275.
51) Katt J, Schwinge D, Schoknecht T, Quaas A, Sobottka I, Burandt E, et al. Increased T helper type 17 response to pathogen stimulation in patients with primary sclerosing cholangitis. Hepatology 2013;58:1084-1093.
52) Bo X, Broome U, Remberger M, Sumitran-Holgersson S. Tumour necrosis factor alpha impairs function of liver derived T lymphocytes and natural killer cells in patients with primary sclerosing cholangitis. Gut 2001;49:131-141.
53) Gao B, Waisman A. Th17 cells regulate liver fibrosis by targeting multiple cell types: many birds with one stone. Gastroenterology 2012;143:536-539.
54) Copple BL, Li T. Pharmacology of bile acid receptors: evolution of bile acids from simple detergents to complex signaling molecules. Pharmacol Res 2016;104:9-21.
55) Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, et al. The gut-liver axis and the intersection with the microbiome. Nat Rev Gastroenterol Hepatol 2018;15:397-411.
56) Perino A, Schoonjans K. TGR5 and Immunometabolism: insights from physiology and pharmacology. Trends Pharmacol Sci 2015;36:847-857.
57) Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. Curr Opin Gastroenterol 2014;30:332-338.
58) Adams DH, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. Nat Rev Immunol 2006;6:244-251.
59) Adams DH, Afford SC. The role of cholangiocytes in the development of chronic inflammatory liver disease. Front Biosci 2002;7:e276-e285.
60) Broomé U, Hultcrantz R, Lefvert AK, Yi Q. Cytokine production from colonic T cells in patients with ulcerative colitis with and without primary sclerosing cholangitis. Dis Colon Rectum 1998;41:1543-1549.
61) Silvain C, Zeevi A, Saidman S, Duquesnoy RJ, Van Thiel DH. Phenotypic and functional characteristics of colonic lymphocytes isolated from patients with primary sclerosing cholangitis and inflammatory bowel disease. Hepatogastroenterology 1995;42:250-258.
62) Gwela A, Siddhanathi P, Chapman RW, Travis S, Powrie F, Arancibia-Cárcamo CV, et al. Th1 and innate lymphoid cells accumulate in primary sclerosing cholangitis-associated inflammatory bowel disease. J Crohns Colitis 2017;11:1124-1134.
63) Ji SG, Juran BD, Mucha S, Folseraas T, Jostins L, Melum E, et al. Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease. Nat Genet 2017;49:269-273.
64) Al Mamari S, Djordjevic J, Halliday JS, Chapman RW. Improvement of serum alkaline phosphatase to <1.5 upper limit of normal predicts better outcome and reduced risk of cholangiocarcinoma in primary sclerosing cholangitis. J Hepatol 2013;58:329-334.
65) Lindström L, Hultcrantz R, Boberg KM, Friis-Liby I, Bergquist A. Association between reduced levels of alkaline phosphatase and survival times of patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol 2013;11:841-846.
66) Tabibian JH, Weeding E, Jorgensen RA, Petz JL, Keach JC, Talwalkar JA, et al. Randomised clinical trial: vancomycin or

metronidazole in patients with primary sclerosing cholangitis—a pilot study. Aliment Pharmacol Ther 2013;37:604-612.
67) Davies YK, Cox KM, Abdullah BA, Safta A, Terry AB, Cox KL. Long-term treatment of primary sclerosing cholangitis in children with oral vancomycin: an immunomodulating antibiotic. J Pediatr Gastroenterol Nutr 2008;47:61-67.
68) Boner AL, Peroni D, Bodini A, Delaini G, Piacentini G. Azithromycin may reduce cholestasis in primary sclerosing cholangitis: a case report and serendipitous observation. Int J Immunopathol Pharmacol 2007;20:847-849.
69) Howden BP, Smith DJ, Mansell A, Johnson PD, Ward PB, Stinear TP, et al. Different bacterial gene expression patterns and attenuated host immune responses are associated with the evolution of low-level vancomycin resistance during persistent methicillin-resistant *Staphylococcus aureus* bacteraemia. BMC Microbiol 2008;8:39.
70) Seifert HA, Benedek G, Nguyen H, Gerstner G, Zhang Y, Kent G, et al. Antibiotics protect against EAE by increasing regulatory and anti-inflammatory cells. Metab Brain Dis 2018;33:1599-1607.
71) Abarbanel DN, Seki SM, Davies Y, Marlen N, Benavides JA, Cox K, et al. Immunomodulatory effect of vancomycin on Treg in pediatric inflammatory bowel disease and primary sclerosing cholangitis. J Clin Immunol 2013;33:397-406.
72) Allegretti JR, Kassam Z, Carrellas M, Timberlake S, Gerardin Y, Pratt D, et al. Fecal microbiota transplantation improves microbiome diversity and liver enzyme profile in primary sclerosing cholangitis. Am J Gastroenterol 2017;112(Suppl.):S539.
73) Vleggaar FP, Monkelbaan JF, van Erpecum KJ. Probiotics in primary sclerosing cholangitis: a randomized placebo-controlled crossover pilot study. Eur J Gastroenterol Hepatol 2008;20:688-692.
74) Poropat G, Giljaca V, Stimac D, Gluud C. Bile acids for primary sclerosing cholangitis. Cochrane Database Syst Rev 2011;CD003626.

Author names in bold designate shared co-first authorship.