

TOPIC HIGHLIGHT

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Etiopathogenesis of primary sclerosing cholangitis

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Abstract

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown etiology but lymphocytic portal tract infiltration is suggestive of an immune-mediated basis for this disease. Associations with inflammatory bowel disease (IBD) especially ulcerative colitis (UC), and with particular autoimmune diseases, as well as the genetic associations further suggest PSC may be an immune-mediated disease. The immunogenetics of PSC have been the subject of active research and several HLA and non-HLA associated genes have been implicated in the development of the disease. Lymphocytes derived from the inflamed gut may enter the liver *via* the enterohepatic circulation to cause hepatic disease. PSC may be triggered in genetically susceptible individuals by infections or toxins entering the portal circulation through a permeable colon and hence evoking an abnormal immune response.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic disease of the intra and/or extrahepatic bile ducts. It is characterized by a concentric obliterative fibrosis

that leads to bile duct strictures (Figure 1). In many, this in turn progresses to biliary cirrhosis and hepatic failure. Approximately one third of patients will develop cholangiocarcinoma^[1]. PSC is frequently associated with inflammatory bowel disease (IBD) usually ulcerative colitis (UC) and those with Crohn's have disease predominantly affecting the colon. Approximately three quarters of the Northern European population with PSC have concomitant IBD particularly extensive UC^[2]. 4.0%-7.5% of patients with UC have PSC^[3].

The term "secondary sclerosing cholangitis (SSC)" is used for a disease with similar clinical features to PSC but where a direct causative agent for the pathological process is known. Such agents include choledocholithiasis with intraductal stones, surgical damage to bile ducts, ischaemia from hepatic artery occlusion, infections, and chemical agents such as drugs. Table 1 comprises a full list of possible causes of SSC with a section also showing the conditions which can mimic sclerosing cholangitis on cholangiography. There is little good data on the natural history of SSC and very little information regarding the immunological processes occurring during the progression of SSC is known although liver biopsies often show similar changes to those of PSC with ductopenia and patchy inflammation. The remainder of this chapter will concentrate on the etiopathogenesis of PSC.

The etiology and pathogenesis of PSC remain very poorly understood. The insidious onset of the disease makes the identification of an aetiological agent very unlikely. As the disease is associated with autoantibodies and HLA haplotypes as well as being closely related to IBD it would appear to be immune mediated. An autoimmune mediated destructive process is also suggested by lymphocytic infiltration into areas of portal damage.

PSC is not however a classical autoimmune disease, as it occurs with a 2:1 male predominance compared with the female predominance found in classical autoimmune diseases such as primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH). Moreover PSC does not have the characteristic response to immunosuppressive treatment as seen in classical autoimmune disease (Table 2).

Circumstantial evidence that PSC may be immune mediated comes from the independent association of PSC with a number of autoimmune diseases. 119 patients with PSC were studied by Saareinen *et al*^[4]. Each



Figure 1 Cholangiogram showing beading and dilatation of the intra and extra hepatic bile ducts- the diagnostic features of primary sclerosing cholangitis (PSC).

Table 1 Causes and mimics of secondary sclerosing cholangitis (SSC)

SSC	
Causes	Surgical trauma to bile ducts Ischaemic injury eg after transplantation Hepatic arterial chemotherapy eg floxuridine Intraductal gallstones ^[3] Viral or bacterial infection eg CMV or cryptosporidiosis Caustic injury eg formalin treatment of hydatid disease Congenital abnormalities eg cystic fibrosis
Conditions mimicking sclerosing cholangitis on imaging	Malignancy eg metastatic carcinoma Hypereosinophilic syndrome Choledochal cyst

Table 2 The features of primary sclerosing cholangitis compared with classical autoimmune disease

Characteristic	Classical autoimmune disease	Immune-mediated inflammatory disease (such as IBD, psoriasis)	Primary sclerosing cholangitis
Age	Children and adults	Children and adults	Children and adults
Sex	Female predominance	No gender predilection	Male predominance
Autoantigens	Yes	No	No
Autoantibodies	Yes (pathogenic)	Yes (markers)	Yes (probably markers)
Associated autoimmune disease	Yes	Yes	Yes
HLA associations (class I and II)	Yes	Yes	Yes
Response to immunosuppression	Usually good	Often good	Good in children Poor in adults

PSC patient with IBD was matched to an IBD patient without PSC; 24% of the PSC patients had one or more autoimmune disorders outside the liver and colon compared with only 9% in the IBD group without PSC. Nine patients in the PSC group had 2 or more autoimmune diseases compared with only 2 in the IBD group. Diabetes mellitus and thyroid diseases were the most common in both groups. It is noteworthy that associated autoimmune disease did not seem to influence the outcome or clinical presentation of PSC^[4].

Simultaneous or sequential occurrence of PSC and AIH has been described in both adult and pediatric populations^[5]. The reported prevalence of this overlap syndrome is variable from 8%-54% and depends on the age of the study population, the type of scoring system used for diagnosis and the completeness of the analysed data.

In general, sclerosing cholangitis in children is characterized by more pronounced autoimmune features with a clinical overlap with AIH. This condition "autoimmune sclerosing cholangitis in childhood" has been addressed elsewhere in this issue (see Miele Verghani).

AUTOANTIBODIES

Atypical anti-neutrophil cytoplasmic antibodies (ANCA) are present in the serum of up to 88% patients with PSC (33%-88%)^[5]. They are however not specific for PSC and are found in UC (60%-87%), and AIH (50%-96%)^[6]. These ANCA are distinct from perinuclear-staining

antineutrophil cytoplasmic antibody (p-ANCA) found in microscopic polyangiitis and cytoplasmic-staining antineutrophil cytoplasmic antibody c-ANCA in Wegener's granulomatosis.

Immunoblotting showed reactivity in 92% of IBD or hepatobiliary disease patients with an atypical p-ANCA to a myeloid specific nuclear protein with a molecular mass of 50 kDa^[7]. The target antigen in PSC for these atypical ANCA is probably a neutrophil nuclear envelope protein, viz tubulin-beta isotype 5^[8]. Terjung and colleagues have suggested that the term p-ANNA is therefore more appropriate as the recognised antigen is not cytoplasmic but originating in the nuclear membrane^[7].

The importance of these autoantibodies in the development of PSC is unknown. Titres of ANCA correlate with disease activity in the systemic vasculitides, whereas in contrast there is a poor correlation between ANCA and clinical parameters in PSC^[9-11]. Titres of ANCA remain unchanged after a transplant in PSC and after a colectomy in UC. Current evidence suggests that they are unlikely to play a role in the pathogenesis of PSC.

A high proportion of non-specific autoantibodies in addition to p-ANNA are found in patients with PSC (Table 3). They are of unclear relevance and unhelpful in diagnosis. These include anti nuclear antibodies (20%-67%), antimitochondrial antibodies (< 10%) and antithyroperoxidase antibodies (7%-16%)^[5]. Anticardiolipin antibodies were found in 66% of PSC patients compared to 4% controls by Angulo but no

Table 3 Autoantibody prevalence in primary sclerosing cholangitis

Antibody	Prevalence (%)
Anti-nuclear antibody (ANA)	7-77
Anti-smooth muscle antibody (ASMA)	13-20
Anti-endothelial cell antibody (AECA)	35
Anti-cardiolipin antibody	4-66
Thyroperoxidase	7-16
Thyroglobulin	4
Rheumatoid factor	15

Anti-mitochondrial antibody is only rarely detected in PSC (< 10%). This is useful in differentiating PSC from primary biliary cirrhosis (PBC). Data taken from Angulo *et al*^[12].

resultant associations with thrombotic disease were demonstrated^[12].

Significantly more PSC patients have autoantibodies to surface antigens expressed on biliary epithelial cells (BEC) than patients with PBC, AIH or normal controls. These induce increased expression of CD44 on the BEC and increased production of IL-6 by BEC^[13]. Anti-BEC autoantibodies may be both IgM and IgG. IL-6 induces BEC proliferation *in vitro* and suppresses BEC apoptosis, and it is increased in the bile in cholangitis and in the serum in cholangiocarcinoma. Persistent IL-6 production may be in part, responsible for the bile duct changes seen in PSC.

Antibodies to the baker's yeast, *Saccharomyces cerevisiae* (ASCA) have been reported in IBD especially active Crohn's disease. ASCA are not autoantibodies but there does seem to be some genetic predisposition to their presence. ASCA has also been seen in autoimmune liver disease including PSC but no conclusions can be drawn from their presence^[14].

IMMUNOGENETICS

PSC is not attributable to one gene locus and is a non-Mendelian (complex) disorder. A number of associations have been made with HLA haplotypes as well as a number of other genes. There is controversy as to whether there is a primary susceptibility allele but PSC is probably acquired through inheriting a combination of genetic polymorphisms that act together to cause susceptibility to disease. The genetics of PSC is still the subject of active research.

Major histocompatibility complex (MHC) genes in PSC

The MHC gene on the short arm of chromosome 6 encodes HLA molecules. Case control association studies have identified various HLA molecules and other immunoregulatory genes as determinants of disease susceptibility and progression in PSC. HLA molecules are highly polymorphic and have a central role in the T cell response. Class I molecules encode HLA A, B and Cw and class II encode the DR, DQ and DP families. The Class III region encodes a number of peptides which are active in the immune response including genes for TNF α and TNF β , complement proteins C4, C2

Table 4 Key HLA haplotypes in PSC^[27]

	HLA haplotypes	Odds ratio
3 HLA haplotypes associated with an increased risk	B8-MICA*008-TNFA*2-DRB3*0101-DRB1*0301-DQB1*0201	2.69
	DRB3*0101-DRB1*1301-DQA1*0103-DQB1*0603	3.8
	MICA*008-DRB5*0101-DRB1*1501-DQA1*0102-DQB1*0602	1.52
3 HLA haplotypes associated with reduced risk (protective)	DRB4*0103-DRB4*0401-DQA1*03-DQB1*0302	0.26
	DRB4*0103-DRB1*0701-DQA1*0201-DQB1*0303	0.15
	MICA*002	0.12

and Bf and MHC class I chain-related (*MICA*) and *MICB* genes encoding the MHC class I chain related molecules α and β . Normal biliary cells express HLA class I and not class II. HLA-DR, DQ and DP are aberrantly expressed on target cells in PSC.

There is an increased frequency of *HLA B8* and *DR3* (*HLA DRB1*0301*) in PSC compared with healthy controls as first described in 1982 and then confirmed in other studies^[15-17]. A later study by Donaldson showed a secondary association with *DR2* in *DR3* negative patients^[18]. An increase in *HLA-DR6* has also been observed in PSC patients^[19,20]. *HLA B8* and *DR3* are in linkage disequilibrium. The *HLA B8*, *DR3* haplotype is also associated with several organ specific autoimmune diseases including lupoid chronic active hepatitis, type I diabetes mellitus, myasthenia gravis and thyrotoxicosis. There is no difference in class II typing between PSC patients with and without autoimmune diseases outside the liver and colon suggesting association of PSC with autoimmune disease is not secondary to HLA but rather a primary phenomenon^[4].

HLA DR4 is less common in PSC than in control populations and the significance of this is disputed^[20]. Studies have suggested that although it has a protective effect against PSC development, when present it is associated with poor prognosis and possibly cholangiocarcinoma^[19,21].

In rheumatoid arthritis (RA) more severe disease has also been seen with certain *DR4* alleles. Gow described the association of RA and PSC in 4 cases^[22]. In three, the liver disease was unusually progressive, proceeding to cirrhosis in 14, 18 and 48 mo from diagnosis. It has been suggested therefore that RA in association with PSC may be a marker of patients at high risk of progression to cirrhosis. PSC also needs to be considered in all RA patients with cholestatic liver tests. The *DR3*, *DR2* heterozygote has been shown to be associated with an increased risk of death or liver transplant and a *DQ6* encoding haplotype in *DR3*, *DR2* negative individuals was associated with a reduced risk^[19].

Molecular genotyping has identified 6 haplotypes that encode for peptides involved in the immune response in PSC (Table 4)^[23].

The finding of multiple haplotypes associated with PSC indicates a complex relationship with the MHC. Susceptibility appears to involve either a combination

of *DR*, *DQ* and *MHC class I chain-like (MIC)* alleles or perhaps *MIC* alone. There is controversy concerning which allele or alleles within each haplotype may form the primary association.

MICA genes are a group of polymorphic genes on chromosome 6. They are localised in the class I region between *HLA-B* and *TNFA*. *MICA* molecules are stress and heat shock inducible and are expressed in non-diseased liver and on thymic and gastrointestinal epithelia. *MICA* has been identified as a ligand for $\gamma\delta$ T cells, natural killer (NK) (CD56+) cells and cells expressing the NKG2D activatory receptor. Increased numbers of both $\gamma\delta$ and NK cells have been documented in PSC livers^[24,25].

An association between the *MICA*008* allele and PSC has been demonstrated by Norris *et al*^[26] (which is due to an increased frequency of patients with 2 copies of this allele (i.e. homozygous). *MICA*008* is the main allele carrying the *MICA5.1* microsatellite allele. PSC has been found to be significantly associated with both the *MICA5.1* and the *MICB24* (*MICB* microsatellite) markers. The association was lost when stratified for *DR3* or *B8* positive and negative individuals. However, *B8* and *DR3* were associated with PSC only in the presence of these markers^[27].

*MICA*002* has a strong negative association with disease and is the functional opposite of *MICA*008*. The *MICA*002* allele carries the *MICA9* microsatellite allele which is also therefore less common in PSC patients compared with controls as this allele has been shown to be protective. One copy of the *MICA*002* allele prevents PSC in most cases and so the resistant allele may be dominant^[26,27].

Bernal first concluded that genetic susceptibility to PSC might be determined by polymorphism within the *TNF* genes^[28]. The *TNF- α* gene is located in the class III HLA region between the *HLA-B* and *DRB3* loci^[29]. Increased frequency of the rare allele *-308A* (termed *TNF2*) of the *TNF* gene promoter has been reported in autoimmune disorders that include RA, systemic lupus erythematosus and coeliac disease. Individuals with this allele may produce high levels of *TNF- α* . *TNF2* is in linkage disequilibrium with the extended *HLA-B8-DR3-DQ2* haplotype. The G to A substitution at position *-308* in the *TNF- α* promoter has been shown by Mitchell *et al* to be associated with susceptibility to PSC, but this was secondary to the association with the *B8-DR3* haplotype^[30].

Non-MHC genes in PSC

HLA haplotypes do not account for all of the susceptibility to develop PSC and genes outside the HLA region may also have a role in disease pathogenesis. Studies of non-MHC genes have failed to show an association between PSC and cytokine genes including *IL-1 β* , *IL-1RN* and *IL-10*^[30,31]. The *CD95 (FAS)* gene (*TNFRSF6*), the gene encoding *CCR-5*, genes encoding *CTLA4* and the *Nod2* gene have also been examined in PSC. Karlsen *et al* have shown that genetic polymorphisms conferring susceptibility to IBD are not found in PSC/IBD patients.

viz *CARD15*, *TLR-4*, *CARD4*, *SLC22A4*, *SLC22A5*, *DLG5* and *MDR1*^[32]. The chemokine receptor-5 (*CCR5*) data are contradictory. *CCR5-Delta32* is a 32 base pair deletion associated with significant reduction in cell surface expression of the receptor. Melum *et al* showed no association of *CCR5-Delta32* with susceptibility or resistance to PSC contradicting earlier reports suggesting an association^[33,34]. Cytotoxic T lymphocyte antigen-4 (*CTLA-4*) is expressed on activated T lymphocytes. It is a cell surface molecule that binds to the ligand CD80 (*B.7*) on antigen presenting cells. A *CTLA-4* gene polymorphism is described in several autoimmune diseases but in PSC this remains in question. The most recent and largest study was unable to demonstrate any effect in PSC^[35].

PSC progression is related to periportal and septal fibrosis and this is associated with excess production and reduced degradation of extracellular matrix. This is regulated by a series of metalloproteinases (MMPs) and their naturally occurring inhibitors. There is a common polymorphism in the promoter sequence of the *stromelysin (MMP3)* gene with either a 5A or 6A repeat. The 5A allele is associated with increased transcription of stromelysin compared to the 6A variant. Satsangi *et al* in Oxford have found an association between the carriage rate of the 5A allele and susceptibility to PSC. 5A homozygosity was associated with development of portal hypertension^[36]. This may suggest the *MMP3* 5A allele as a marker for fibrosis.

Wienke *et al* could not confirm the association of the *MMP-3 5A* allele with PSC and also found no general associations of the *MMP-1* promoter polymorphism among Norwegian patients^[37]. Patients with PSC who also had UC were found however to have an increased frequency of the *MMP-3* allele *5A* compared with PSC patients without UC (60% compared to 45%). All patients with cholangiocarcinoma were found to be carriers of the *MMP-1* allele *1G* compared with 72% of those with PSC who did not have cholangiocarcinoma.

Intracellular adhesion molecule-1 (ICAM-1, CD54) gene polymorphisms have been implicated in the susceptibility to a number of inflammatory conditions, including IBD. In PSC, studies have found that patients with advanced disease express ICAM on proliferating bile ductules and interlobular bile ducts. Increased soluble ICAM levels have been found in the serum of patients with PSC probably indicating activation of the immune system and inflammatory responses^[38]. Yang *et al* have shown recently that, in British patients, the *ICAM-1* polymorphism K469E is associated with PSC and may be a protective allele. This association is independent of the coexistence of IBD. There is no relationship between the *ICAM-1* genotype and the rate of PSC progression^[39]. These results were not confirmed in a Scandinavian population^[40].

CELLULAR IMMUNE ABNORMALITIES IN PSC

There is a T cell predominant portal infiltrate in PSC

although the relative proportions and importance of the CD4 and CD8 cells are not known. CD4 cells are seen more commonly in the portal tracts and CD8 cells predominate in areas of interface hepatitis^[41]. The cell infiltrate may change as the disease progresses. These cells are functional and are likely to be involved in the pathogenesis of disease. In the peripheral circulation there does appear to be a fall in CD8 cells as the disease progresses. This only occurs late in disease so is unlikely to be significant in disease pathogenesis^[41-44].

Bo and colleagues showed that cell proliferation and function of liver derived T lymphocytes is impaired in PSC patients compared with liver derived T cells obtained from normal controls or patients with other autoimmune liver diseases^[45]. They believe this is due to exposure to high levels of TNF *in vivo* and this exposure may be chronic.

Previously relatively high levels of TNF- α have been seen in T cell lines from liver biopsies in patients with different stages of PSC while decreased levels were observed in PBC patients. Therefore increased levels of TNF- α are present in PSC patients whether the disease be early or late stage^[45-46].

T cells in PSC

PSC is characterized by a prominent T cell infiltrate in areas of portal damage. The T cell receptor (TCR) determines the specificity of T cells. It consists of two disulphide linked polypeptides, α and β . An alternative receptor, namely $\gamma\delta$ has been identified. The predominant cell type is still $\alpha\beta$ and the significance of T cells with $\gamma\delta$ in PSC is not known^[42]. TCR genes show genetic diversity but the $V\alpha\beta$ gene segment of the TCR can play a dominant role in recognition of certain peptide-MHC complexes. Expanded T cell populations using restricted sets of TCR V gene segments have been identified in areas of inflammation in the tissues affected in other immunopathic diseases such as RA and Sjogren's disease^[47]. Broome reported the preferential expression in liver tissue of the $V\beta 3$ region of the T cell receptor in PSC patients compared with liver tissue from PBC patients and healthy controls but no differences were seen in peripheral blood T cells^[48]. This may indicate the presence of a specific antigen in the liver in PSC patients capable of driving the T cell production with this $V\beta 3$ segment.

Oligoclonal T cell receptors that proliferate in culture with enterocytes and are cytotoxic to enterocyte cell lines were reported in PSC but this study is unconfirmed^[49]. There are to date no studies of regulatory T cells (T regs) in PSC patients.

In summary, the available data do not as yet allow for any useful hypothesis on the T-cell contribution to the lesions of PSC.

BEC

BEC appear to act as the target for the immune response in PSC and are also an active participant in the immune reaction. They express a number of cytokines, enzymes, intracellular adhesion molecules (ICAM-1) and HLA

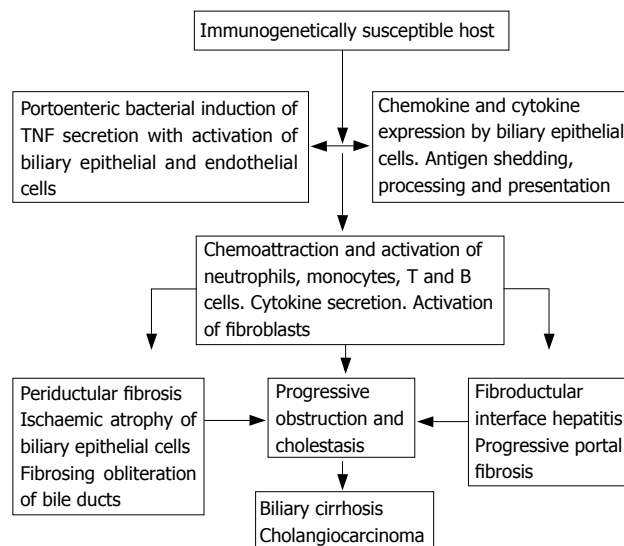


Figure 2 Vierling's hypothesis of the pathogenesis of primary sclerosing cholangitis^[72].

molecules. Normal BEC express only HLA class I and not class II whereas there is aberrant expression of class II molecules on BEC in PSC^[50-52], and also in PBC. Functionally important autoantibodies have been found to antigens on BEC in PSC. These induce BECs to produce IL-6 and increased expression of CD44. BEC however seem to lack the co stimulatory molecules necessary to activate T cells and unstimulated BEC inhibit T cell activation and this casts doubt upon the theory that BECs can act as antigen presenting cells^[53,54].

However, it has become clear that cholangiocytes rather than being passive targets may play primary roles in the pathogenesis of peribiliary inflammation and periductular fibrosis in PSC^[54,55]. Stimulation by proinflammatory cytokines induces cholangiocyte secretion of multiple chemokines, cytokines, and growth factors that immunomodulate inflammation and fibrogenesis^[55]. The chemoattracted T cells include a population of PSC-specific T cells primed in the gut.

BACTERIA IN PSC

The association between PSC and IBD led to Vierling's hypothesis that colonic bacteria enter the portal circulation through a leaky mucosa in IBD thereby causing PSC (Figure 2)^[55].

Bacterial antigens may act as molecular mimics in genetically susceptible people and cause an immune reaction responsible for initiating PSC. The bacteria are able to get through gut walls made permeable by colitis or in theory by any infective episode of acute infective or inflammatory colitis. Chemokines and cytokines are then released from Kupffer cells in the liver attracting macrophages/monocytes, lymphocytes, activated neutrophils and fibroblasts to the portal tracts. Vierling further suggested that the concentric fibrosis resulting could cause atrophy of the BEC secondary to ischaemia. The bile duct loss would lead to progressive cholestasis, further fibrosis and secondary biliary

cirrhosis. This does not explain however why there are fewer PSC patients with Crohn's colitis as compared with UC and why there can be an associated stricturing of the pancreatic duct.

Portal bacteremia has been described in UC patients undergoing colectomy^[56]. A study looking at explanted livers showed higher bacterial positivity rates in bile and bile ducts in PSC patients compared with PBC patients, and α -haemolytic streptococci accounted for 46% of the bacterial strains found. Bile duct cannulation at endoscopic retrograde cholangiopancreatography (ERCP) could have accounted for this bacterial presence^[57]. The study went on therefore to compare patients with PSC who had undergone ERCP to those who had not, in order to evaluate the potential role of these bacteria in the etiopathogenesis of PSC. Positive cultures were obtained from 3 of the naive PSC patients and from 6 of the PSC patients with previous ERCP. α -haemolytic streptococci were again the commonest bacteria seen. As most naive PSC patients were found to have negative bacterial cultures this bacteria is unlikely to play a primary role in etiopathogenesis but may be involved in disease progression^[58].

Recent molecular studies have shown an increased prevalence of *H pylori* and other non-gastric *Helicobacter species* in cholestatic liver diseases compared with healthy controls and noncholestatic liver disease. In PSC positivity was significantly but weakly associated with UC^[59].

Ponsioen *et al* have suggested an association between PSC and previous Chlamydia infection after the finding of an increase in seroprevalence of Chlamydia anti-lipopolysaccharide (LPS) antibodies in PSC patients, although no Chlamydia antibodies were found in liver tissue and thus the significance is unclear^[60].

Among animal models, none has yet have been developed showing all the features of PSC, although a rat model in which there is small bowel bacterial overgrowth has shown hepatic injury somewhat similar to that seen in human PSC^[61,62].

Abnormal accumulation of lipopolysaccharide (a bacterial endotoxin), presumably derived from portal blood, in the biliary epithelium has been shown in a rat model with a self-filling blind intestinal loop, and therefore may be involved in the pathogenesis of bile duct injury associated with intestinal injury^[63]. Are these studies very persuasive?

LYMPHOCYTE HOMING

PSC is strongly linked to IBD but it also runs a course independent from the bowel disease illustrated by the fact that the disease can develop many years after colectomy. Grant *et al* hypothesized that T lymphocytes generated in the gut during active inflammation persist as long-lived memory cells and undergo enterohepatic circulation and can then trigger an inflammatory response in the liver when activated by an appropriate stimulus. The nature of the stimulus remains unclear; possibilities include hepatic expression of the original priming antigen or possibly mediation solely by the

aberrant expression of gut specific adhesion molecules and chemokines^[64].

There is overlapping expression of many molecules between the gut and liver including the two potential addressins vascular adhesion protein-1 (VAP-1) and mucosal addressin cell adhesion molecule-1 (MAdCAM-1). VAP-1 expression on liver endothelium is normally far stronger than that seen on mucosal vessels. In IBD gut expression is greatly increased, suggesting that lymphocytes from the liver may be able to enter the inflamed gut using VAP-1. MAdCAM-1 endothelial expression was thought to be restricted to the gut but has been recently seen on portal endothelium in inflammatory liver disease (including PSC) associated with IBD. Mucosal lymphocytes express $\alpha_4\beta_7$, which allows adhesion to hepatic MAdCAM-1 suggesting it may play a role in lymphocyte recruitment^[65-67]. They propose that memory lymphocyte cells recirculate between liver and gut using either/both MAdCAM-1 and VAP-1^[65].

The chemokine CCL21 activates lymphocyte adhesion to MAdCAM-1 dependent on $\alpha_4\beta_7$. CCL21, thought to only exist in secondary lymphoid tissue is upregulated in portal associated lymphoid tissue in PSC and plays an important role in recruiting lymphocytes. Expression of the gut-associated chemokine CCL25 (thymus-expressed chemokine (TECK)) has also been shown in PSC liver sinusoidal endothelium but was absent in liver in AIH/PBC. A significant population of CCR9+ mucosal lymphocytes (capable of binding CCL25) has been detected infiltrating PSC liver tissue compared with controls and matched peripheral blood, thus supporting the hypothesis of a T cell enterohepatic recirculation. CCR9 lymphocytes co-express the gut homing integrin $\alpha_4\beta_7$. Therefore CCL25 recruits CCR9+ lymphocytes to the liver in PSC by triggering adhesion to MAdCAM-1^[66-67]. MAdCAM-1 and CCL25 are upregulated to the liver in inflammatory liver diseases whereas previously they were thought to be restricted to the gut. Conversely VAP-1, normally expressed in the liver, is up regulated in the gut in IBD^[68].

However this does not explain why PSC is associated more with UC than Crohn's disease, as it would be predicted that just as many memory T cells are produced in Crohn's disease as in UC.

Hepatobiliary transporters in PSC

Defects in the hepatobiliary transport system have been shown to be the cause of a number hereditary cholestatic disorders eg progressive familial intrahepatic cholestasis and BSEP (bile salt export pump)^[69]. This system is responsible for the hepatocellular uptake and excretion of bile salts into bile canaliculi. Defects in the transport system can result in bile duct injury.

Knockout mice for the *Mdr2 (Abcb4)* gene, which corresponds to human *MDR3/ABCB4*, spontaneously develop sclerosing cholangitis with features similar to human PSC^[70]. A non-functional multidrug resistance 3 (MDR3) protein leads to the formation of a "toxic" bile with increased concentration of free, non-micellar bile acids which cause BEC injury, pericholangitis, periductal fibrosis and, eventually, sclerosing cholangitis. Studies in

Table 5 Comparison of PSC and AIP-SC

	PSC	AIP-SC
Gender	M:F = 2:1	Probably some male predominance ^[81,85,87]
Clinical presentation	Usually insidious. Sometimes with obstructive jaundice secondary to cholangiocarcinoma.	Mild abdo/Back pain Sometimes with short history of obstructive jaundice due to CBD stricture
Associated inflammatory bowel disease	Yes	No
Cholangiographic findings	Diffuse changes throughout intra- and extrahepatic bile ducts. Abnormalities in pancreatic duct common.	Pancreatic duct strictures or narrowing. Often stricture of distal 1/3 of common bile duct. Intrahepatic duct changes less common.
Blood chemistry data	Often cholestatic but bilirubin usually near normal.	May be cholestatic. Bilirubin often high
Autoantibodies	Atypical pANCA plus range of others	Antibodies to carbonic anhydrase II plus range of others ^[80,81,84]
Immunoglobulins	IgG4 levels normal	IgG4 levels usually elevated ^[82]
Histology	Absence of plasma cells positive for IgG4 on immunostaining	IgG4 positive plasma cells present in bile ducts and portal tracts ^[79]
Liver biopsy staging	Range of Ludwig staging including higher stages eg III or IV	Ludwig staging usually only I or II ^[86]
Treatment	Ursodeoxycholic acid ± biliary drainage for dominant strictures	Systemic steroid therapy usually leads to complete resolution of symptoms and signs of disease. Occasionally patients relapse and require longer courses of steroids

Table 6 Evidence for the influence of immune mechanisms on the aetiology of PSC

	Evidence for the influence of immune mechanisms
Humoral immunity	Increased circulating immune complexes Elevated immunoglobulin levels (IgG and IgM) Low titres of non-organ specific autoantibodies (ANA and SMA) High titres of antineutrophil nuclear antibody (ANNA)
Cell mediated immunity	Decreased levels of circulating peripheral CD8+ve T cells Portal T cell and NK cell infiltrate Increased activated and memory T cells Restricted T cell receptor repertoire (Vβ3) Aberrant expression of HLA-DR on BEC Coexpression of costimulatory molecules and HLA-DR on BECs Abnormal expression of adhesion molecules on biliary epithelial cells Abnormal expression of chemokine ligands on biliary epithelial cells
Immune effector mechanisms	Enhanced cytokine expression in the liver
Immunogenetic mechanisms	HLA associations

PSC patients, however, did not find MDR3 variations^[71]. Similarly, the role of the cystic fibrosis transmembrane conductance regulator (CFTR) remains controversial^[72-74]. The potential role of other hepatobiliary transporters eg BSEP, AE2 in the pathogenesis of PSC remains to be explored. As defects in these systems are known to cause bile duct injury and cholangitis, they are excellent candidates for further investigation.

The nuclear receptor SXR is a nuclear bile acid receptor which plays an important role in endogenous bile acid homeostasis and cholesterol synthesis. A recent study of PSC patients has shown that functional SXR gene variants modify the disease progression and affect survival^[75].

Autoimmune pancreatitis (IgG4 associated sclerosing cholangitis)

Sarles *et al*^[6] in 1961 provided the first description of what was later identified as autoimmune pancreatitis, an increasingly recognised benign inflammatory disease of the pancreas^[77]. Abnormalities and sclerosing changes in both the intra- and extra hepatic bile ducts are well recognised in AIP (see pp this issue), and can cause diagnostic confusion with PSC. Correct diagnosis is important as AIP responds well to corticosteroid therapy and tends to have a significantly better outcome than PSC^[71-81]. The association of AIP and sclerosing changes in the bile ducts has been termed AIP-SC^[81-85]. Diagnostic criteria for AIP have been proposed and developed by the Japan Pancreas society^[86]. These criteria consist of the finding of a diffuse narrowing of the pancreatic duct on imaging studies, and either a laboratory finding of an abnormally elevated serum gamma globulin, IgG, or more particularly IgG4 or the presence of autoantibodies or classical histopathological features of the disease ie fibrotic changes with a lymphocyte and, characteristically, plasma cell infiltration. The differences between the two conditions are summarised in Table 5.

CONCLUSION

Immune mechanisms play an important role in the pathogenesis of PSC, although it remains unclear whether it is a classical autoimmune disease (Tables 2 and 6). There are strong MHC genetic associations including HLA molecules and the MIC molecules. HLA haplotypes however do not account for all the genetic susceptibility in the development of PSC and there is uncertainty about the importance of genes outside this region. Bacterial antigens may act as molecular mimics in hosts who are genetically susceptible and therefore cause an immune reaction leading to PSC initiation.

Lymphocytes may move from the inflamed gut in IBD *via* the enterohepatic circulation and cause inflammation of the liver when activated by a specific stimulus such as bacterially derived antigens.

REFERENCES

- 1 **Worthington J**, Cullen S, Chapman R. Immunopathogenesis of primary sclerosing cholangitis. *Clin Rev Allergy Immunol* 2005; **28**: 93-103
- 2 **Aadland E**, Schrumpf E, Fausa O, Elgjo K, Heilo A, Aakhus T, Gjone E. Primary sclerosing cholangitis: a long-term follow-up study. *Scand J Gastroenterol* 1987; **22**: 655-664
- 3 **Broome U**, Bergquist A. Primary sclerosing cholangitis, inflammatory bowel disease, and colon cancer. *Semin Liver Dis* 2006; **26**: 31-41
- 4 **Saarinen S**, Olerup O, Broome U. Increased frequency of autoimmune diseases in patients with primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**: 3195-3199
- 5 **Levy C**, Lindor KD. Primary sclerosing cholangitis: epidemiology, natural history, and prognosis. *Semin Liver Dis* 2006; **26**: 22-30
- 6 **Terjung B**, Worman HJ. Anti-neutrophil antibodies in primary sclerosing cholangitis. *Best Pract Res Clin Gastroenterol* 2001; **15**: 629-642
- 7 **Terjung B**, Spengler U, Sauerbruch T, Worman HJ. "Atypical p-ANCA" in IBD and hepatobiliary disorders react with a 50-kilodalton nuclear envelope protein of neutrophils and myeloid cell lines. *Gastroenterology* 2000; **119**: 310-322
- 8 **Terjung B**, Muennich M, Gottwein J, Soehne J. Identification of myeloid-specific tubulin-beta isotype 5 as target antigen of antineutrophil cytoplasmic antibodies in autoimmune liver disease. *Hepatology* 2005; **42**: 288A
- 9 **Mulder AH**, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CG. Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver diseases. *Hepatology* 1993; **17**: 411-417
- 10 **Bansi DS**, Bauducci M, Bergqvist A, Boberg K, Broome U, Chapman R, Fleming K, Jorgensen R, Lindor K, Rosina F, Schrumpf E. Detection of antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis: a comparison of the alkaline phosphatase and immunofluorescent techniques. *Eur J Gastroenterol Hepatol* 1997; **9**: 575-580
- 11 **Pokorny CS**, Norton ID, McCaughan GW, Selby WS. Antineutrophil cytoplasmic antibody: a prognostic indicator in primary sclerosing cholangitis. *J Gastroenterol Hepatol* 1994; **9**: 40-44
- 12 **Angulo P**, Peter JB, Gershwin ME, DeSotel CK, Shoenfeld Y, Ahmed AE, Lindor KD. Serum autoantibodies in patients with primary sclerosing cholangitis. *J Hepatol* 2000; **32**: 182-187
- 13 **Xu B**, Broome U, Ericzon BG, Sumitran-Holgersson S. High frequency of autoantibodies in patients with primary sclerosing cholangitis that bind biliary epithelial cells and induce expression of CD44 and production of interleukin 6. *Gut* 2002; **51**: 120-127
- 14 **Muratori P**, Muratori L, Guidi M, Maccariello S, Pappas G, Ferrari R, Gionchetti P, Campieri M, Bianchi FB. Anti-Saccharomyces cerevisiae antibodies (ASCA) and autoimmune liver diseases. *Clin Exp Immunol* 2003; **132**: 473-476
- 15 **Chapman RW**, Varghese Z, Gaul R, Patel G, Kokinin N, Sherlock S. Association of primary sclerosing cholangitis with HLA-B8. *Gut* 1983; **24**: 38-41
- 16 **Shepherd HA**, Selby WS, Chapman RW, Nolan D, Barbatis C, McGee JO, Jewell DP. Ulcerative colitis and persistent liver dysfunction. *Q J Med* 1983; **52**: 503-513
- 17 **Schrumpf E**, Fausa O, Forre O, Dobloug JH, Ritland S, Thorsby E. HLA antigens and immunoregulatory T cells in ulcerative colitis associated with hepatobiliary disease. *Scand J Gastroenterol* 1982; **17**: 187-191
- 18 **Donaldson PT**, Farrant JM, Wilkinson ML, Hayllar K, Portmann BC, Williams R. Dual association of HLA DR2 and DR3 with primary sclerosing cholangitis. *Hepatology* 1991; **13**: 129-133
- 19 **Farrant JM**, Doherty DG, Donaldson PT, Vaughan RW, Hayllar KM, Welsh KI, Eddleston AL, Williams R. Amino acid substitutions at position 38 of the DR beta polypeptide confer susceptibility to and protection from primary sclerosing cholangitis. *Hepatology* 1992; **16**: 390-395
- 20 **Spurkland A**, Saarinen S, Boberg KM, Mitchell S, Broome U, Caballeria L, Ciusani E, Chapman R, Ercilla G, Fausa O, Knutsen I, Pares A, Rosina F, Olerup O, Thorsby E, Schrumpf E. HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. *Tissue Antigens* 1999; **53**: 459-469
- 21 **Mehal WZ**, Lo YM, Wordsworth BP, Neuberger JM, Hubscher SC, Fleming KA, Chapman RW. HLA DR4 is a marker for rapid disease progression in primary sclerosing cholangitis. *Gastroenterology* 1994; **106**: 160-167
- 22 **Gow PJ**, Fleming KA, Chapman RW. Primary sclerosing cholangitis associated with rheumatoid arthritis and HLA DR4: is the association a marker of patients with progressive liver disease? *J Hepatol* 2001; **34**: 631-635
- 23 **Donaldson PT**. Genetics of liver disease: immunogenetics and disease pathogenesis. *Gut* 2004; **53**: 599-608
- 24 **Hata K**, Van Thiel DH, Herberman RB, Whiteside TL. Phenotypic and functional characteristics of lymphocytes isolated from liver biopsy specimens from patients with active liver disease. *Hepatology* 1992; **15**: 816-823
- 25 **Martins EB**, Graham AK, Chapman RW, Fleming KA. Elevation of gamma delta T lymphocytes in peripheral blood and livers of patients with primary sclerosing cholangitis and other autoimmune liver diseases. *Hepatology* 1996; **23**: 988-993
- 26 **Norris S**, Kondeatis E, Collins R, Satsangi J, Clare M, Chapman R, Stephens H, Harrison P, Vaughan R, Donaldson P. Mapping MHC-encoded susceptibility and resistance in primary sclerosing cholangitis: the role of MICA polymorphism. *Gastroenterology* 2001; **120**: 1475-1482
- 27 **Wiencke K**, Spurkland A, Schrumpf E, Boberg KM. Primary sclerosing cholangitis is associated to an extended B8-DR3 haplotype including particular MICA and MICB alleles. *Hepatology* 2001; **34**: 625-630
- 28 **Bernal W**, Moloney M, Underhill J, Donaldson PT. Association of tumor necrosis factor polymorphism with primary sclerosing cholangitis. *J Hepatol* 1999; **30**: 237-241
- 29 **Donaldson PT**, Norris S. Immunogenetics in PSC. *Best Pract Res Clin Gastroenterol* 2001; **15**: 611-627
- 30 **Mitchell SA**, Grove J, Spurkland A, Boberg KM, Fleming KA, Day CP, Schrumpf E, Chapman RW. Association of the tumour necrosis factor alpha -308 but not the interleukin 10 -627 promoter polymorphism with genetic susceptibility to primary sclerosing cholangitis. *Gut* 2001; **49**: 288-294
- 31 **Donaldson PT**, Norris S, Constantini PK, Bernal W, Harrison P, Williams R. The interleukin-1 and interleukin-10 gene polymorphisms in primary sclerosing cholangitis: no associations with disease susceptibility/resistance. *J Hepatol* 2000; **32**: 882-886
- 32 **Karlsen TH**, Hampe J, Wiencke K, Schrumpf E, Thorsby E, Lie BA, Broome U, Schreiber S, Boberg KM. Genetic polymorphisms associated with inflammatory bowel disease do not confer risk for primary sclerosing cholangitis. *Am J Gastroenterol* 2007; **102**: 115-121
- 33 **Eri R**, Jonsson JR, Pandeya N, Purdie DM, Clouston AD, Martin N, Duffy D, Powell EE, Fawcett J, Florin TH, Radford-Smith GL. CCR5-Delta32 mutation is strongly associated with primary sclerosing cholangitis. *Genes Immun* 2004; **5**: 444-450
- 34 **Melum E**, Karlsen TH, Broome U, Thorsby E, Schrumpf E, Boberg KM, Lie BA. The 32-base pair deletion of the

- chemokine receptor 5 gene (CCR5-Delta32) is not associated with primary sclerosing cholangitis in 363 Scandinavian patients. *Tissue Antigens* 2006; **68**: 78-81
- 35 **Wiencke K**, Boberg KM, Donaldson P, Harbo H, Ling V, Schrupf E, Spurkland A. No major effect of the CD28/CTLA4/ICOS gene region on susceptibility to primary sclerosing cholangitis. *Scand J Gastroenterol* 2006; **41**: 586-591
- 36 **Satsangi J**, Chapman RW, Halidar N, Donaldson P, Mitchell S, Simmons J, Norris S, Marshall SE, Bell JL, Jewell DP, Welsh KI. A functional polymorphism of the stromelysin gene (MMP-3) influences susceptibility to primary sclerosing cholangitis. *Gastroenterology* 2001; **121**: 124-130
- 37 **Wiencke K**, Louka AS, Spurkland A, Vatn M, Schrupf E, Boberg KM. Association of matrix metalloproteinase-1 and -3 promoter polymorphisms with clinical subsets of Norwegian primary sclerosing cholangitis patients. *J Hepatol* 2004; **41**: 209-214
- 38 **Bloom S**, Fleming K, Chapman R. Adhesion molecule expression in primary sclerosing cholangitis and primary biliary cirrhosis. *Gut* 1995; **36**: 604-609
- 39 **Yang X**, Cullen SN, Li JH, Chapman RW, Jewell DP. Susceptibility to primary sclerosing cholangitis is associated with polymorphisms of intercellular adhesion molecule-1. *J Hepatol* 2004; **40**: 375-379
- 40 **Bowlus CL**, Karlsen TH, Broome U, Thorsby E, Vatn M, Schrupf E, Lie BA, Boberg KM. Analysis of MAdCAM-1 and ICAM-1 polymorphisms in 365 Scandinavian patients with primary sclerosing cholangitis. *J Hepatol* 2006; **45**: 704-710
- 41 **Hashimoto E**, Lindor KD, Homburger HA, Dickson ER, Czaja AJ, Wiesner RH, Ludwig J. Immunohistochemical characterization of hepatic lymphocytes in primary biliary cirrhosis in comparison with primary sclerosing cholangitis and autoimmune chronic active hepatitis. *Mayo Clin Proc* 1993; **68**: 1049-1055
- 42 **Lindor KD**, Wiesner RH, Katzmann JA, LaRusso NF, Beaver SJ. Lymphocyte subsets in primary sclerosing cholangitis. *Dig Dis Sci* 1987; **32**: 720-725
- 43 **Whiteside TL**, Lasky S, Si L, Van Thiel DH. Immunologic analysis of mononuclear cells in liver tissues and blood of patients with primary sclerosing cholangitis. *Hepatology* 1985; **5**: 468-474
- 44 **Snook JA**, Chapman RW, Sachdev GK, Heryet A, Kelly PM, Fleming KA, Jewell DP. Peripheral blood and portal tract lymphocyte populations in primary sclerosing cholangitis. *J Hepatol* 1989; **9**: 36-41
- 45 **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
- 46 **Spengler U**, Moller A, Jung MC, Messer G, Zachoval R, Hoffmann RM, Eisenburg J, Paumgartner G, Riethmuller G, Weiss EH. T lymphocytes from patients with primary biliary cirrhosis produce reduced amounts of lymphotoxin, tumor necrosis factor and interferon-gamma upon mitogen stimulation. *J Hepatol* 1992; **15**: 129-135
- 47 **Imberti L**, Sottini A, Primi D. T cell repertoire and autoimmune diseases. *Immunol Res* 1993; **12**: 149-167
- 48 **Broome U**, Grunewald J, Scheynius A, Olerup O, Hultcrantz R. Preferential V beta3 usage by hepatic T lymphocytes in patients with primary sclerosing cholangitis. *J Hepatol* 1997; **26**: 527-534
- 49 **Probert CS**, Christ AD, Saubermann LJ, Turner JR, Chott A, Carr-Locke D, Balk SP, Blumberg RS. Analysis of human common bile duct-associated T cells: evidence for oligoclonality, T cell clonal persistence, and epithelial cell recognition. *J Immunol* 1997; **158**: 1941-1948
- 50 **Broome U**, Glaumann H, Hultcrantz R, Forsum U. Distribution of HLA-DR, HLA-DP, HLA-DQ antigens in liver tissue from patients with primary sclerosing cholangitis. *Scand J Gastroenterol* 1990; **25**: 54-58
- 51 **Chapman RW**, Kelly PM, Heryet A, Jewell DP, Fleming KA. Expression of HLA-DR antigens on bile duct epithelium in primary sclerosing cholangitis. *Gut* 1988; **29**: 422-427
- 52 **Van den Oord JJ**, Sciort R, Desmet VJ. Expression of MHC products by normal and abnormal bile duct epithelium. *J Hepatol* 1986; **3**: 310-317
- 53 **Cruickshank SM**, Southgate J, Selby PJ, Trejdosiewicz LK. Inhibition of T cell activation by normal human biliary epithelial cells. *J Hepatol* 1999; **31**: 1026-1033
- 54 **Leon MP**, Bassendine MF, Wilson JL, Ali S, Thick M, Kirby JA. Immunogenicity of biliary epithelium: investigation of antigen presentation to CD4+ T cells. *Hepatology* 1996; **24**: 561-567
- 55 **O'Mahony CA**, Vierling JM. Etiopathogenesis of primary sclerosing cholangitis. *Semin Liver Dis* 2006; **26**: 3-21
- 56 **Brooke BN**, Dykes PW, Walker FC. A study of liver disorder in ulcerative colitis. *Postgrad Med J* 1961; **37**: 245-251
- 57 **Olsson R**, Bjornsson E, Backman L, Friman S, Hockerstedt K, Kaijser B, Olausson M. Bile duct bacterial isolates in primary sclerosing cholangitis: a study of explanted livers. *J Hepatol* 1998; **28**: 426-432
- 58 **Bjornsson ES**, Kilander AF, Olsson RG. Bile duct bacterial isolates in primary sclerosing cholangitis and certain other forms of cholestasis--a study of bile cultures from ERCP. *Hepatogastroenterology* 2000; **47**: 1504-1508
- 59 **Nilsson HO**, Taneera J, Castedal M, Glatz E, Olsson R, Wadstrom T. Identification of Helicobacter pylori and other Helicobacter species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. *J Clin Microbiol* 2000; **38**: 1072-1076
- 60 **Ponsoen CY**, Defoer J, Ten Kate FJ, Weverling GJ, Tytgat GN, Pannekoek Y, Wertheim-Dillen PM. A survey of infectious agents as risk factors for primary sclerosing cholangitis: are Chlamydia species involved? *Eur J Gastroenterol Hepatol* 2002; **14**: 641-648
- 61 **Lichtman SN**, Sartor RB, Keku J, Schwab JH. Hepatic inflammation in rats with experimental small intestinal bacterial overgrowth. *Gastroenterology* 1990; **98**: 414-423
- 62 **Vierling JM**. Animal models for primary sclerosing cholangitis. *Best Pract Res Clin Gastroenterol* 2001; **15**: 591-610
- 63 **Koga H**, Sakisaka S, Yoshitake M, Harada M, Kumemura H, Hanada S, Taniguchi E, Kawaguchi T, Kumashiro R, Sata M. Abnormal accumulation in lipopolysaccharide in biliary epithelial cells of rats with self-filling blind loop. *Int J Mol Med* 2002; **9**: 621-626
- 64 **Grant AJ**, Lalor PF, Salmi M, Jalkanen S, Adams DH. Homing of mucosal lymphocytes to the liver in the pathogenesis of hepatic complications of inflammatory bowel disease. *Lancet* 2002; **359**: 150-157
- 65 **Eksteen B**, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hubscher SG, Briskin M, Salmon M, Adams DH. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. *J Exp Med* 2004; **200**: 1511-1517
- 66 **Grant AJ**, Lalor PF, Hubscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). *Hepatology* 2001; **33**: 1065-1072
- 67 **Eksteen B**, Miles AE, Grant AJ, Adams DH. Lymphocyte homing in the pathogenesis of extra-intestinal manifestations of inflammatory bowel disease. *Clin Med* 2004; **4**: 173-180
- 68 **Eksteen B**, Curbishley SM, Lai WK, Adams DH. Liver dendritic cells in primary sclerosing cholangitis (PSC) are unable to imprint mucosal adhesion molecules in primed lymphocytes without exogenous retinoic acid. *J Hepatol* 2006; **44**: S10 (Abstract)
- 69 **Strautnieks SS**, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, Sokal E, Dahan K, Childs S, Ling V, Tanner MS, Kagalwalla AF, Nemeth A, Pawlowska J, Baker A, Mieli-Vergani G, Freimer NB, Gardiner RM, Thompson RJ. A

- gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. *Nat Genet* 1998; **20**: 233-238
- 70 **Fickert P**, Fuchsbichler A, Wagner M, Zollner G, Kaser A, Tilg H, Krause R, Lammert F, Langner C, Zatloukal K, Marschall HU, Denk H, Trauner M. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* 2004; **127**: 261-274
- 71 **Pauli-Magnus C**, Kerb R, Fattinger K, Lang T, Anwald B, Kullak-Ublick GA, Beuers U, Meier PJ. BSEP and MDR3 haplotype structure in healthy Caucasians, primary biliary cirrhosis and primary sclerosing cholangitis. *Hepatology* 2004; **39**: 779-791
- 72 **Gallegos-Orozco JF**, E Yurk C, Wang N, Rakela J, Charlton MR, Cutting GR, Balan V. Lack of association of common cystic fibrosis transmembrane conductance regulator gene mutations with primary sclerosing cholangitis. *Am J Gastroenterol* 2005; **100**: 874-878
- 73 **Sheth S**, Shea JC, Bishop MD, Chopra S, Regan MM, Malmberg E, Walker C, Ricci R, Tsui LC, Durie PR, Zielenski J, Freedman SD. Increased prevalence of CFTR mutations and variants and decreased chloride secretion in primary sclerosing cholangitis. *Hum Genet* 2003; **113**: 286-292
- 74 **Girodon E**, Sternberg D, Chazouilleres O, Cazeneuve C, Huot D, Calmus Y, Poupon R, Goossens M, Housset C. Cystic fibrosis transmembrane conductance regulator (CFTR) gene defects in patients with primary sclerosing cholangitis. *J Hepatol* 2002; **37**: 192-197
- 75 **Karlsen TH**, Lie BA, Frey Frosli K, Thorsby E, Broome U, Schrumpf E, Boberg KM. Polymorphisms in the steroid and xenobiotic receptor gene influence survival in primary sclerosing cholangitis. *Gastroenterology* 2006; **131**: 781-787
- 76 **Sarles H**, Sarles JC, Muratore R, Guien C. Chronic inflammatory sclerosis of the pancreas--an autonomous pancreatic disease? *Am J Dig Dis* 1961; **6**: 688-698
- 77 **Erkelens GW**, Vleggaar FP, Lesterhuis W, van Buuren HR, van der Werf SD. Sclerosing pancreato-cholangitis responsive to steroid therapy. *Lancet* 1999; **354**: 43-44
- 78 **Kim KP**, Kim M, Lee YJ, Song MH, Park DH, Lee SS, Seo DW, Lee SK, Min YI, Song DE, Yu ES. [Clinical characteristics of 17 cases of autoimmune chronic pancreatitis] *Korean J Gastroenterol* 2004; **43**: 112-119
- 79 **Uehara T**, Hamano H, Kawa S, Sano K, Honda T, Ota H. Distinct clinicopathological entity 'autoimmune pancreatitis-associated sclerosing cholangitis'. *Pathol Int* 2005; **55**: 405-411
- 80 **Ito T**, Nakano I, Koyanagi S, Miyahara T, Migita Y, Ogoshi K, Sakai H, Matsunaga S, Yasuda O, Sumii T, Nawata H. Autoimmune pancreatitis as a new clinical entity. Three cases of autoimmune pancreatitis with effective steroid therapy. *Dig Dis Sci* 1997; **42**: 1458-1468
- 81 **Okazaki K**. Autoimmune pancreatitis: etiology, pathogenesis, clinical findings and treatment. The Japanese experience. *JOP* 2005; **6** Suppl 1: 89-96
- 82 **Hamano H**, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, Fukushima M, Nikaide T, Nakayama K, Usuda N, Kiyosawa K. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001; **344**: 732-738
- 83 **Okazaki K**, Uchida K, Chiba T. Recent concept of autoimmune-related pancreatitis. *J Gastroenterol* 2001; **36**: 293-302
- 84 **Uchida K**, Okazaki K, Konishi Y, Ohana M, Takakuwa H, Hajiro K, Chiba T. Clinical analysis of autoimmune-related pancreatitis. *Am J Gastroenterol* 2000; **95**: 2788-2794
- 85 **Zamboni G**, Luttges J, Capelli P, Frulloni L, Cavallini G, Pederzoli P, Leins A, Longnecker D, Kloppel G. Histopathological features of diagnostic and clinical relevance in autoimmune pancreatitis: a study on 53 resection specimens and 9 biopsy specimens. *Virchows Arch* 2004; **445**: 552-563
- 86 **Nakazawa T**, Ohara H, Sano H, Ando T, Aoki S, Kobayashi S, Okamoto T, Nomura T, Joh T, Itoh M. Clinical differences between primary sclerosing cholangitis and sclerosing cholangitis with autoimmune pancreatitis. *Pancreas* 2005; **30**: 20-25
- 87 **Kamisawa T**, Yoshiike M, Egawa N, Nakajima H, Tsuruta K, Okamoto A. Treating patients with autoimmune pancreatitis: results from a long-term follow-up study. *Pancreatol* 2005; **5**(2-3): 234-238; discussion 8-40.

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