Immunology of Primary Sclerosing Cholangitis

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Abbreviations

aa	Amino acid
Ags	Antigens
AID	Autoimmune disease
APCs	Antigen-presenting cells
AutoAbs	Autoantibodies
AutoAg	Autoantigen
BSEP	Bile salt export protein
C'	Complement
CCL	Chemokine ligand for CC chemokine receptors
CCR5Δ32	Chemokine receptor 5 with 32 base pair deletion
CD	Crohn's disease
CFTR	Cystic fibrosis transmembrane conductance regulator
CpG	Bacterial dinucleotide PAMP
CTL	Cytotoxic T lymphocyte
CTLA4	Cytotoxic T lymphocyte antigen-4
DCs	Dendritic cells
ERCP	Endoscopic retrograde cholangiopancreatography
FISH	Fluorescence in situ hybridization
GALT	Gut-associated lymphoid tissue
HLA	Human leukocyte antigen complex (designation for human MHC)
IBD	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule-1 (CD54)
IFNγ	Interferon gamma
IL	Interleukin
IMID	Immune-mediated inflammatory disease
LPS	Lipopolysaccharide
MADCAM-1	Mucosal vascular addressin cell adhesion molecule-1
Mdr2	Multidrug resistance gene product 2, the mouse homolog of human MDR3
MDR3	Multidrug resistance gene product 3, a human bile transporter
MHC	Major histocompatibility complex
MMPs	Matrix metalloproteinases
NK cell	Natural killer cell
NKT	Natural killer T cell
NSDC	Nonsuppurative destructive cholangitis
OLT	Orthotopic liver transplantation
PAMPs	Pathogen-associated molecular patterns
pANCAs	Perinuclear antineutrophil cytoplasmic antibodies
pANNAs	Peripheral antineutrophil nuclear antibodies
PBMC	Peripheral blood mononuclear cells
PDGF	Platelet-derived growth factor
PRRs	Pattern recognition receptors

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PSC	Primary sclerosing cholangitis
TCRs	T cell receptors for peptide antigens
TGFβ	Transforming growth factor-beta
Th	T helper
TLRs	Toll-like receptors
TNFα	Tumor necrosis factor-alpha
Treg	Regulatory CD4 T cell
UC	Ulcerative colitis
VAP-1	Vascular adhesion protein-1
VCAM-1	Vascular cell adhesion molecule-1
Vβ	Variable region of β-chain of TCR

Introduction

Primary sclerosing cholangitis (PSC) is a rare, chronic, progressive hepatobiliary disease of undefined etiology that affects macroscopic intrahepatic and/or extrahepatic bile ducts in the majority and microscopic proximal bile ducts in a minority (<10 %) [1, 2]. PSC is associated with inflammatory bowel disease (IBD) of the colon in >75 % of cases; ulcerative colitis (UC) of a distinctive phenotype afflicts the majority and Crohn's disease (CD) the minority [3]. In PSC, peribiliary inflammation results in progressive circumferential fibrosis causing biliary strictures. Currently, PSC is classified as an "atypical" autoimmune disease (AID) because several features of PSC differ from those of a classical AID (Table 9.1) [4].

A form of secondary sclerosing cholangitis associated with elevations of serum IgG4 and/or IgG4-secreting B and plasma cells may mimic PSC [5]. Retrospective studies indicate that approximately 10 % of patients diagnosed with PSC instead may have IgG4 cholangiopathy [6]. IgG4 cholangiopathy can be distinguished by a prior history of pancreatitis, stricturing of both intrahepatic and extrahepatic bile ducts, propensity for jaundice, and the use of recently developed techniques [7].

Multiple immunological features suggest involvement of innate and adaptive immune responses in immunopathogenesis, including susceptibility and resistance associations with HLA haplotypes and autoantibodies (autoAbs), and evidence that gut-primed T effector T cells mediate peribiliary, fibrosing inflammation [4, 8]. The homing and retention of these gut-primed T cells are facilitated by the activated cholangiocytes that

Table 9.1 Comparison of characteristic features of classical autoimmune diseases and primary sclerosing cholangitis

Features	Classical AID	PSC
Autoantigen(s)	Yes	Possibly
Autoantibody	Yes, pathogenetic	Yes, biomarker
Age	Children and adults	Children and adults
Gender predilection	Female > male	Male > female
Genetic factors	HLA, non-HLA	HLA, non-HLA
Tissue- or organ-specific disease	Yes	Yes
Inflammatory cells	Autoreactive T cells	Gut-primed T cells, NK, NKT, macrophages, γδ T cells
Environmental factors	Yes	Yes
Associated AIDs	Yes	Yes
Response to immunosuppression	Yes	No
Examples	SLE Myasthenia gravis Graves' disease Pernicious anemia Type 1 diabetes AIH PBC	

Abbreviations: *AID* autoimmune disease, *HLA* human major histocompatibility complex, *NK* natural killer cells, *NKT* natural killer T cells, *SLE* systemic lupus erythematosus, *AIH* autoimmune hepatitis, *PBC* primary biliary cholangitis

express ligands and receptors and secretion of inflammatory cytokines and chemokines [9]. Thus, the cholangiocytes are not passive targets of the immune response but participate in the immunopathogenesis of PSC [4].

The goal of this chapter is to provide a progress report on the immunology of PSC. Emphasis is placed on immunological findings advancing our understanding of the immunopathogenesis of PSC.

Biliary Anatomic Features and PSC

The branching network of bile ducts is lined by cholangiocytes with tight junctions that retain bile within the duct lumens (Fig. 9.1) [10, 11]. Each bile duct is accompanied by a branch of the hepatic artery of equal caliber that gives rise to a peribiliary capillary plexus surrounding each duct. Lymphatic channels adjacent to the peribiliary capillaries drain lymph formed in the space of Disse that contains cytokines and other constituents produced in the hepatic lobules. The portal venous blood from the small bowel and colon contains pathogen-associated molecular patterns (PAMPs) from the cell walls and unmethylated DNA of gut bacteria and fungi, metabolites produced by the gut microbiota, and viable microbial pathogens when the gut mucosal barrier is breached. PSC markedly alters these homeostatic anatomic relationships.

Pathology of PSC

The histopathology of PSC is unique among primary biliary tract diseases (Fig. 9.2) [12]. Lymphoplasmacytic infiltrates of the portal tracts localize to the peribiliary space, where they promote peribiliary fibrosis without apoptotic destruction of the cholangiocytes. The density of portal inflammation is scant, especially when compared to either autoimmune hepatitis (AIH) or primary biliary cholangitis (PBC). A key feature distinguishing PSC from PBC is the absence of effector cell-mediated apoptosis of cholangiocytes in PSC [13].

Progressive fibrosis leads to concentric, circumferential laminations around intact intrahe-

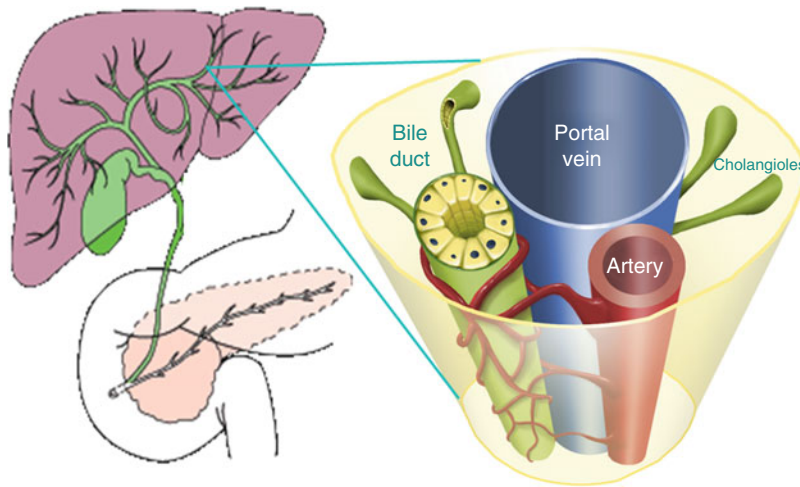

Fig. 9.1 Biliary anatomic features involved in primary sclerosing cholangitis. An intralobular bile duct receives the bile secreted by hepatocytes through cholangioles at the periphery of the portal tract. Each intrahepatic bile duct is accompanied by a branch of the hepatic artery of equal caliber. The arteries supply a peribiliary capillary plexus surrounding each duct, while lymphatic channels lie adjacent to the peribiliary capillaries and drain lymph formed in the space of Disse that contains cytokines and other constituents produced in the hepatic lobules. The portal venous blood from the small bowel and colon contains pathogen-associated molecular patterns (PAMPs) from the cell walls and unmethylated DNA of gut bacteria and fungi, metabolites produced by the gut microbiota, and viable microbial pathogens when the gut mucosal barrier is breached

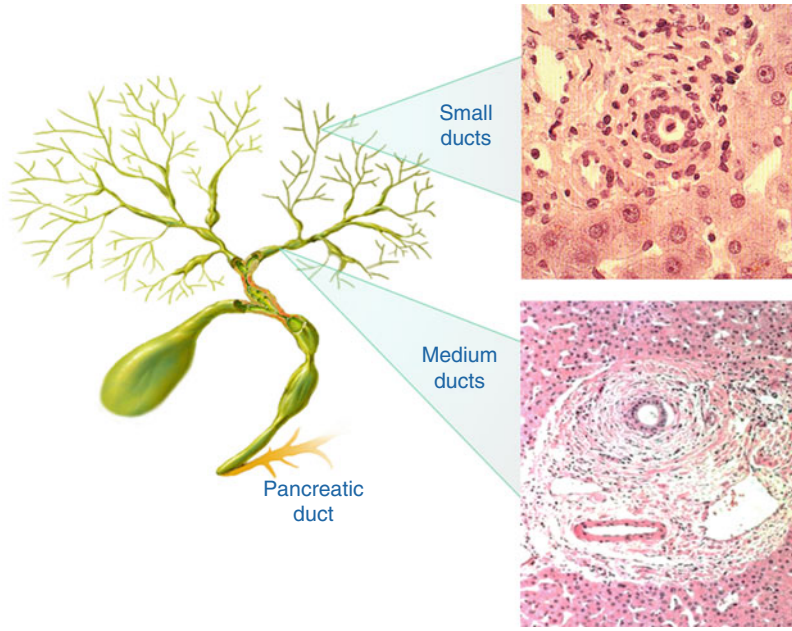

Fig. 9.2 Histopathology of small duct and medium duct primary sclerosing cholangitis. The histopathology of PSC includes the small duct variant and the fibrous inflammatory lesions of medium-caliber intrahepatic ducts. Compared to either AIH or PBC, the inflammatory infiltrates in PBC are sparse. Periductal, concentric fibrosis of the medium-caliber intralobular bile ducts pushes the peribiliary capillary plexi away from the basement membranes of the bile ducts

patic bile ducts, referred to as "onion skin" fibrosis, that displace the peribiliary capillary plexi, creating a physical and spatial barrier to oxygenation and maintenance of the cholehepatic countercurrent circulation between the bile duct and artery [14]. Thus, the pathogenesis of stricturing, circumferential peribiliary fibrosis also involves relative arterial or capillary ischemia. Stimuli of periductal fibrosis include secretion of chemokines and cytokines by innate immune cells and activated cholangiocytes and the inflammatory and fibrotic response to toxic bile leaking between injured cholangiocytes [4, 15]. Proinflammatory cytokines and/or microbial molecules in lymph or blood induce cholangiocyte expression of chemokine receptors and secretion of chemokines and cytokines involved in the chemoattraction of effector cells to the peribiliary space and promotion of fibrogenesis [4, 16].

Innate and Adaptive Immunity

Innate Immunity

Innate immunity provides immediate reactions against microbial pathogens and cells altered by stress, infection, or neoplasia [17, 18]. Innate immune responses are mediated by macrophages (including Kupffer cells), dendritic cells (DCs), natural killer (NK), and NKT cells. Macrophages and DCs constitutively express pattern recognition receptors (PRRs) for invariant microbial molecules, collectively called PAMPs, and for CD14 and activated complement (C') molecules. Toll-like receptors (TLRs) are the most prominent PRRs, expressed on innate immune cells and epithelial cells, including cholangiocytes and hepatocytes. Since PAMPs are molecular fragments of microbes, innate immune responses do not require viable microbes. PAMPs relevant to the immunopathogenesis of PSC [11, 19] include (1) lipopolysaccharide (LPS, aka endotoxin), the signature cell wall component of all Gram-negative bacteria; (2) lipoteichoic acid, the signature cell wall component of Gram-positive bacteria; (3) peptidoglycans, essential cell wall components of all bacteria; and (4) unmethylated, bacterial CpG dinucleotide motifs. Class I chain-related MICA and MICB genes encode ligands expressed by cells damaged by stress, infection, or neoplasia that bind to NKG2D receptors on NK cells, NKT cells, macrophages, and γδT cells causing target cell lysis. In addition, MICA ligands also costimulate CD8 CTLs through their NKG2D receptors.

Innate Immunity in PSC

Intense, unregulated innate immune responses are involved in PSC immunopathogenesis [4, 20]. The cholangiocytes of PSC patients express normal amounts of TLR4 and nucleotide-binding oligomerization domain-containing protein (NOD)-like receptor family pyrin domain-containing 3 (NLRP3) but excessive TLR9 [21]. TLR9 expression correlated with fibrosis stages and greater risk for orthotopic liver transplantation (OLT). Cholangiocytes activated by TLRs, proinflammatory cytokines, and interferon-γ (IFNγ) produce cytokines and chemokines involved in the peribiliary localization of specific inflammatory cells and peribiliary fibrogenesis (Fig. 9.3, discussed below) [4, 9, 22].

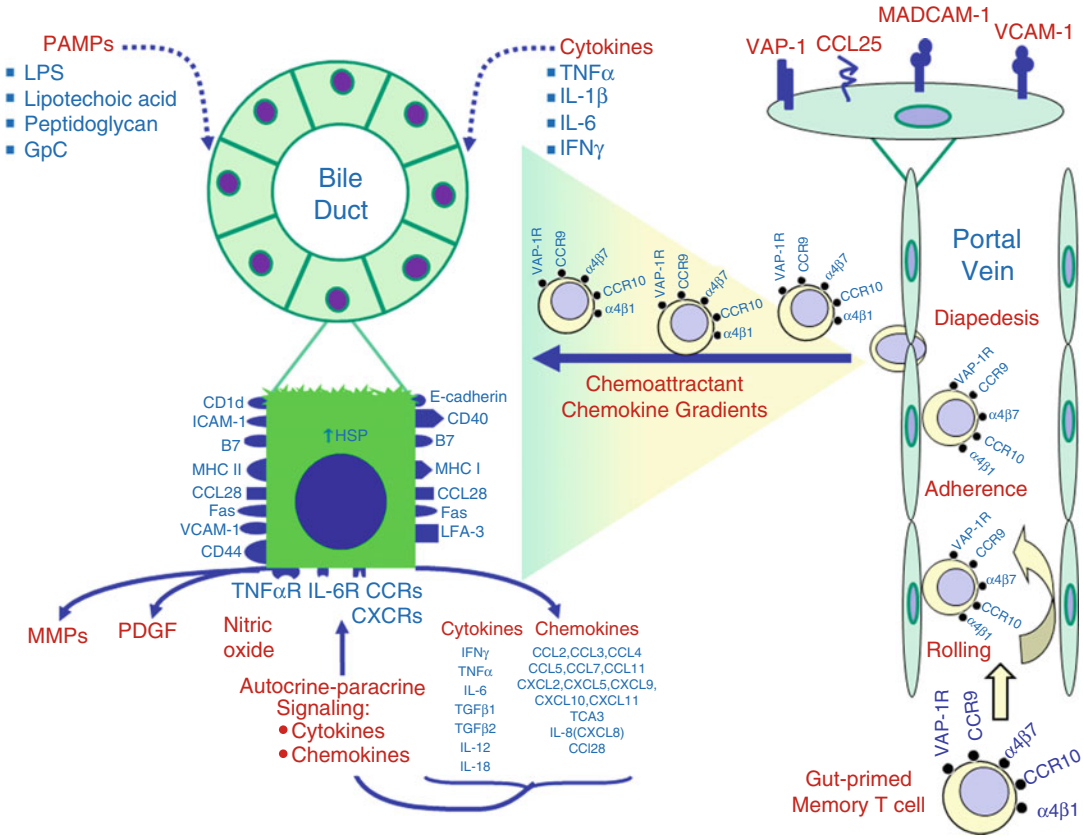

Fig. 9.3 Activated cholangiocytes and gut-primed T cells in the immunopathogenesis of primary sclerosing cholangitis. Cholangiocytes are activated by PAMPs and by proinflammatory cytokines TNFα, IL-1β, IL-6, and IFNγ. Activated gene expression leads to cholangiocyte production of multiple immunological ligands and receptors, chemokines, cytokines, MMPs, PDGF, NO, and aberrant class II HLA. In PSC, cholangiocytes secrete the chemokine CCL25, the ligand for CCR9 on gut-primed T cells. Portal endothelial cells in PSC livers express VAP-1, whose amine oxidase function in the presence of proinflammatory cytokines, especially TNFα, results in aberrant expression of MADCAM-1 and display of CCL25. This permits adhesion and diapedesis of gut-primed memory T cells bearing the α1β7 integrin receptor for MADCAM-1 and the chemokine receptor CCR9 for the CCL25. After transendothelial migration, gut-primed memory T cells migrate along the gradients of chemokines secreted by activated cholangiocytes to congregate in the peribiliary space. The chemokine CCL28 facilitates peribiliary recruitment of T cells bearing its chemokine receptor, CCR10, while VCAM-1 on the cell surface of cholangiocytes acts as ligand for the T cell integrin receptor α1β4. This postulated scheme does not require the presence of gut Ag(s) that originally primed the T cells in the GALT. The absence of cholangiocyte expression of the priming gut Ag(s) may explain the observation that peribiliary T cells do not cause apoptosis of cholangiocytes in PSC

Adaptive Immunity

Adaptive immunity involves delayed immune responses of T cell receptors (TCRs) to processed peptide antigens (Ags, potentially including autoAgs) presented within Ag-binding grooves of class I and II major histocompatibility complex molecules (MHC, designated HLA in humans) expressed by professional antigen-presenting cells (APCs) [23, 24]. Professional APCs include DCs of the innate immune system and activated B cells. CD4 T cell TCRs react with processed exogenous Ags presented by class II MHC molecules and stimulate Ag-specific CD4 T cell TCRs, while CD8 TCRs are stimulated by endogenous (including viral) Ags presented by class I MHC molecules. MHC binding of specific peptide Ags is genetically determined [25, 26]. The non-polymorphic MHC class I-like molecule, CD-1, presents lipid Ags to TCRs expressed by γδT cells. γδT cells are involved in mucosal immunity, surveillance of neoplastic changes, and protection from autoimmune diseases and microbial infections [27]. Class III MHC genes encode TNFα/β; C' factors C4, C2, and Bf; as well as heat shock proteins [25, 26].

HLA

HLA genes are inherited from each parent to form haplotype pairs [25, 26]. Class I HLA, expressed by HLA-A, HLA-B, and HLA-Cw loci, presents peptide Ags to TCRs of cytotoxic CD8 T cells. Class II HLA, expressed by HLA-DR, HLA-DQ, and HLA-DP loci, presents processed peptide Ags to TCRs of CD4 T cells. Polymorphic HLA class I and II Ag-binding grooves determine whether binding and presentation of specific peptide Ags occur, thus conferring susceptibility or resistance to development of a disease like PSC. The class III locus encodes polymorphic immune response proteins, including TNFα/β, complement (C') factors, and heat shock proteins.

Effector T Cells and Cytokines

Ag activation of CD4 T helper (Th) cells triggers exclusive pathways of differentiation that generate Ag-specific Th1, Th2, Th17, Th9, and T follicular helper (Tfh) cells and T regulatory (Treg) subsets [28]. A milieu containing IL-12, IL-18, and INFγ favors CD4 differentiation into Th1 cells that secrete the signature cytokines of Th1 cells: IL-2, INFγ, and TNFα/β. Th1 cytokines provide help for proliferation and differentiation of CD8 T cells, also called cytotoxic T lymphocytes (CTLs), and activate macrophages. Th1 cytokines also induce B cell secretion of C'-fixing IgG2a. In contrast, a milieu containing IL-4 favors CD4 differentiation into Th2 that secretes the signature cytokine profile of Th2 cells, IL, and activates eosinophils and mast cells. The signature cytokines of CD4 Th1 inhibit the proliferation of Th2 cells and vice versa, creating a dynamic balance between Th1 and Th2 subsets within inflammatory infiltrates. Transforming growth factor-beta (TGFβ), IL-6, IL-21, IL-23, and retinoic acid receptor-related orphan receptors γ and α (RORγ, RORα) promote generation of Th17 cells that can become either protective or pathogenic. Both Th1 IFNγ and Th2 IL-4 inhibit Th17 differentiation. Pathogenic Th17 cells are induced by IL-23 and IL-1β to secrete IL-17A, IL-17F, IL-21, and IL-22. In autoimmunity, Th17 effector cells intensify and perpetuate tissue inflammation. Th9 cells have not been evaluated in PSC; however, several functions indicate that they may be relevant to immunopathogenesis [29]. For example, secretion of IL-9 increases gut permeability, activates mast cells, and increases leukocyte recruitment. Th9 cells also secrete IL-21, which promotes IFNγ production by NK cells and CD8 T cells, and IL-3, which enhances DC survival. Tfh cells localize within B cell follicles in lymph nodes and Peyer's patches, where they promote selection and survival of B cell clones by expression of CD40 ligand and secretion of IL-4 and IL-21 [30].

CD4 Treg cells mediate Ag-specific suppression of T cell responses by local secretion of IL-10 and transforming growth factor-beta (TGFβ) [28]. The protective Th17 subset of Treg17 cells is induced by IL-6 and TGFβ.

Adaptive Immunity in PSC

Recent studies have focused on the role and functions of Tregs in PSC. Genome-wide association

studies (GWAS) identified single nucleotide polymorphisms (SNPs) that could affect Treg cells, which led to studies of circulating and hepatic quantities of $CD4^{+}$-$CD25^{high}$-$FOXP3^{+}$-$CD127^{low}$ Tregs [31]. Tregs were significantly decreased in the blood and liver, and their suppressor function was reduced. Reduced Tregs in the blood significantly correlated with homozygosity for the major allele of the SNP rs10905718 in the IL-2RA gene. These findings provide a genetic basis for immune dysregulation caused by reduced Treg numbers in PSC. Another study of Tregs in peripheral blood mononuclear cells (PBMC) of patients with concurrent PSC and UC showed higher frequencies of Tregs compared to those in patients with UC alone [32].

Among the autoAbs associated with PSC is an IgA anti-cholangiocyte Ab, which occurs at high frequency and is correlated with more rapid progression to death or OLT compared to PSC patients without this autoAb [33]. The signature cytokine of Th17 cells, IL-17A, promotes hepatic inflammation and fibrosis [34]. To investigate Th17 immune responses to pathogens in PSC, hepatic bile obtained using endoscopic retrograde cholangiopancreatography (ERCP) was cultured, and liver biopsies were stained using 16sRNA fluorescence in situ hybridization (FISH) [34]. The bile grew multiple bacterial and fungal species and FISH detected microbes in 12 of 13 (92%) of portal tracts. Stimulation PBMC with microbes cultured from the bile generated high frequencies of Th17 cells, especially in response to *Candida albicans*. Th17 cells expressing IL-17A were detected in the peribiliary space, indicating a pathogenic role in the generation of fibrosing inflammation.

Transendothelial Leukocyte Trafficking into Tissues

Activated, circulating leukocytes enter tissues by a multistep process of transendothelial migration [8, 9, 16]. Cellular injury or stress causes secretion of chemokines that are taken up by endothelial cells and displayed on their luminal surfaces along with adhesion molecules. As circulating, activated leukocytes expressing chemokine receptors and counter-receptors for adhesion molecules encounter activated endothelial cells, their leukocyte selectin receptors cause them to roll along the endothelium. Rolling ceases when firm leukocyte adhesion occurs due to binding of leukocyte chemokine receptors to chemokines displayed by endothelial cells and leukocyte integrin adhesion molecules to endothelial cellular adhesion molecules. This initiates diapedesis of leukocytes through endothelial tight junctions and basement membranes into the tissue, where they are chemoattracted along the chemokine gradient toward the source of chemokine secretion. Thus, both chemokines and adhesion molecules expressed on the endothelium determine the composition of inflammatory infiltrates entering the tissue from the blood. As discussed below, this process appears to play a key role in the immunopathogenesis of PSC [8, 9, 16].

Progress Toward an Understanding of Immunopathogenesis

Genetics

Genome-Wide Association Studies (GWAS)

Genetic susceptibility to PSC was assessed in a GWAS of 443,816 single nucleotide polymorphisms (SNPs) in 285 Norwegian PSC patients and 298 healthy controls [35]. Detected associations were reassessed in independent case-control panels in 766 PSC patients and 2,935 controls from Scandinavia, Belgium, the Netherlands, and Germany. The strongest associations were near the HLA-B locus (rs3099844, OR −4.8, 95% CI 3.6–6.5, $p = 2.6 \times 10^{-26}$, and rs2844559, OR 4.7, 95% CI 3.5–6.4, $p = 4.2 \times 10^{-26}$). Non-HLA rs9524260 on chromosome 13q31 was significantly associated with three of four groups. This locus encodes glycan 6, and inhibition of glycan 6 in a cholangiocyte cell line resulted in upregulation of proinflammatory markers.

Subsequent dense genotyping of 130,422 SNPs in immune-related disease regions was performed in 3,789 PSC patients of European ancestry and compared with 2,079 controls [36]. In addition to confirming three significant non-HLA associa-

tions, nine new non-HLA associations were detected. Six of the nine were more strongly associated with PSC than with comorbid IBD. These studies have expanded the genetic risk map of PSC, providing a better understanding of the relationship of PSC and other immune-mediated diseases.

Fucosyltransferase 2 (FUT2)

FUT2 introduces fucose into glycoproteins and glycolipids. FUT2 activity influences interactions between the host and microbes [37]. The nonsense mutation G428A and missense mutation A385T are the principal variants that cause 20% of people to be FUT2 "nonsecretors," incapable of secreting fucose-containing Ags and lacking epithelial cell fucosylation. GWAS indicated that inactivating FUT2 variants were associated with PSC, Crohn's disease, and biochemical markers of biliary injury [37]. The microbiome of nonsecretors was characterized by reduced bifidobacteria, increased *Firmicutes*, and decreased *Proteobacteria* and *Actinobacteria*. The bacterial content of the bile also differed from that of secretors. Lack of fucosylated glycans on the surface of cholangiocytes is potentially deleterious because it would interrupt the glycocalyx required for the protective biliary bicarbonate umbrella that shields cholangiocytes from hydrophobic bile salt toxicity.

HLA and Susceptibility to PSC

PSC susceptibility is most strongly associated with four distinct HLA haplotypes (Table 9.2) [35, 38–41]. The highest susceptibility is conferred by homozygosity for MICA*008 (OR 5.01), suggesting that this allele is closely linked to a true susceptibility allele [42]. The MICA*008 allele contains the MICA5.1 microsatellite allele, which explains the microsatellite's significant association with PSC. It is possible that the NKG2D ligand produced by the MICA*008 allele might explain the increased numbers of NK and γδT cells in PSC livers [43, 44]. The MICB microsatellite allele MICB24 is also significantly associated with PSC. Of note, PSC associations with both MICA5.1 and MICB24 microsatellites are observed exclusively with the HLA-B8-DR3 haplotype [45].

Table 9.2 Immunogenetic associations of PSC with HLA and non-HLA alleles

Susceptibility haplotypes	*Odds ratio*
B8-MICA*008-TNFA*2-DRB3*0101-DRB1*0301-DQA1*0501 DQB1*0201	2.69
DRB3*0101-DRB1*1301-DQA1*0103-DQB1*0603	3.80
MICA*008-DRB5*0101-DRB1*1501-DQA1*0102-DQB1*0602	1.52
(MICA*008 homozygosity)	5.01
Resistance haplotypes	
DRB4*-DRB1*0401-DQA1*0301-DQB1*0302	0.26
DRB4*-DRB1*0701-DQA1*0201-DQB1*0303	0.15
MICA*002	0.12
Non-MHC associations	
ICAM-1	NA
MMP-1, MMP-3	NA
CTLA4	NA
CCR5Δ32 deletion	NA
CFTR	NA

The fact that the HLA-DR3 haplotype is absent from the other two HLA haplotypes associated with the second greatest susceptibility risk (OR 3.80) has been interpreted as evidence of linkage disequilibrium among HLA-B8, MICA*008, TNFα promoter (TNFA*2), and a yet unidentified susceptibility allele. Since DRB1 alleles are present in all three extended susceptibility HLA haplotypes, V or G at position 86 of the DRβ chain was analyzed. V86 was associated with susceptibility alleles DRB1*0301, DRB1*1301, and DRB1*1501 (OR 3.01), while G86 was associated with resistance alleles DRB1*0401 and DRB1*04 (OR 0.17). Modeling of susceptibility and resistance indicated that K87 and P55 in the DQB also could explain susceptibility (OR 2.78) or resistance (OR 0.28).

Of interest, one of the HLA susceptibility haplotypes contains the TNFA*2 allele (Table 9.2). Autoimmunity is associated with TNF-2 allele -308A [46], but a G-308A substitution in the TNFα promoter is linked with susceptibility only with the DRB3*0101 haplotype [47]. PSC susceptibility was not associated with the A to G polymorphism of Fas (encoded by the TNFSF6 gene) [48].

A single HLA susceptibility allele may exist in PSC, but it is more likely that PSC susceptibility is genetically complex, involving multiple HLA and non-HLA SNPs. Currently, PSC susceptibility can be explained for only 50 % of PSC cases on the basis of any allele, amino acid substitutions in the DRβ peptide, or homozygosity for MICA*008 [38]. This is independent of IBD, since UC is unassociated with these HLA haplotypes or MICA*008. Further investigations will require studies of SNPs identified in GWAS.

Susceptibility associations of HLA-DR3 and class III TNFA*2 and the G-308A substitution in the TNFα promoter may explain the association of PSC with AIDs [49]. HLA-DR3$^+$ leukocytes secrete significantly greater amounts of IL-2, IL-5, IL-12, and IFNγ than do HLA-DR3$^-$ leukocytes, before and after mitogen stimulation in vitro [50]. In contrast, HLA-DR3 haplotype does not influence secretion of anti-inflammatory Th2 cytokines IL-4 or IL-10. Susceptibility for PSC may reflect overproduction of TNFα and IFNγ. If high levels of these cytokines are obligatory for immunopathogenesis, it would be plausible that patients capable of generating similar levels of cytokines might develop PSC in the absence of HLA-DR3.

Non-MHC Genes and Susceptibility to PSC

Polymorphic non-HLA gene products involved in inflammation and immunoregulation may be biomarkers of progression and severity of PSC. No susceptibility associations have been identified for Nod2, IL-1, IL-1B, and IL-RN [19, 48]. CTLA4, a T cell receptor for costimulatory B7 ligands that downregulates T cell activation, is of great interest, since CTLA4 polymorphisms increase the risk of multiple organ-specific AIDs [51]. Susceptibility for PSC remains controversial, being present in one study and not in another [48]. The mutant chemokine receptor 5 with a deletion of 32 base pairs (CCR5Δ32) has reduced expression and function. Although initial results were controversial, a recent study showed that PSC susceptibility was significantly associated with CCR5Δ32 [52]. Fibrosis results from a dynamic imbalance between matrix metalloproteinases (MMPs) and inhibitors of metalloproteinases. The MMP-3 gene, encoding stromelysin, exhibits a promoter sequence polymorphism (5A or 6A repeat). A 5A allelic association was observed in one study but was not confirmed in another [53, 54]. The 5A allele was found more frequently in PSC patients with UC (60 %) than in PSC alone (45 %) [54]. The MMP-9 polymorphism R279Q was significantly associated with susceptibility [55]. No association was noted with MMP-1 promoter polymorphisms [54]. The TGFB1 gene encoding the profibrotic and immunosuppressive cytokine TGFβ was not associated with PSC [48]. The absence of the murine bile transporter, Mdr2 (Abcb4), caused regurgitation of toxic bile through leaky cholangiocyte tight junctions, resulting in PSC-like lesions. In contrast, PSC is characterized by normal bile acid transporter haplotypes for MDR3 (human homolog of murine Mdr2), ABCB4, and bile salt export protein (BSEP) ABCB11; thus, there is no evidence of a susceptibility association [56]. Of note, claudin-1 gene mutations compromise tight junctions and are associated with neonatal ichthyosis and sclerosing cholangitis [57]. PSC-like lesions in cystic fibrosis prompted testing for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). One report indicated an increased prevalence of CFTR mutations and defective nasal CFTR Cl^- channel function [58], but others failed to confirm these findings [59]. Induction of experimental colitis in cftr−/− knockout mice did cause PSC-like lesions, suggesting that CFTR mutations might contribute to pathogenesis of PSC in the presence of active IBD [60].

MHC Genes and Resistance to PSC

Three HLA haplotypes reduce the risk of PSC (Table 9.2). HLA-DR4 is the most protective; however, when PSC occurs in HLA-DR4-positive patients, they paradoxically have poorer prognosis and an increased risk of cholangiocarcinoma [61]. One copy of either the MICA*002 allele or its satellite allele MICA9 also confers significant resistance [42, 45]. Given the strong susceptibility risk of PSC bestowed by MICA*008, the resistance association with MICA*002 strongly suggests that MICA-encoded ligands for the NKG2D receptors of innate immune-responsive cells and CD8 CTLs

are determinants of the immunopathogenesis of PSC. MICA allelic associations also imply involvement of innate immune effector cells and microbial PAMPs in immunopathogenesis.

Non-MHC Genes and Resistance to PSC

PSC patients have significantly lower frequencies for both ICAM-1 (CD54)-E469E homozygosity and its extended G241-E469/G241-E469 haplotype [62]. E469E homozygosity may protect against PSC by altering the adhesion required for transendothelial migration and target cell engagement. Resistance occurs with or without coexistent IBD.

Immunogenetics of Disease Progression and Complications of PSC

HLA and non-HLA alleles appear to be involved in PSC progression, severity, and complications. A study of HLA class II alleles in 265 PSC patients from five European countries reported that heterozygosity for the DRB1*03-DQA1*0501-DQB1*02 (HLA-DR3, HLA-DR2 extended haplotype) significantly increased the risk of death or liver transplantation (HR 1.63, 95% CI 1.06–2.52) [63]. In the absence of HLA-DR3 and HLA-DR2, a HLA-DQ6 allele encoding DQB1*0603 or DQB1*0602 significantly reduced both risks (HR 0.57, 95% CI 0.36–0.88). HLA-DR4 and HLA-DQ8 showed a nonsignificant trend for an increased risk of cholangiocarcinoma. The CCR5Δ32 genotype was more prevalent in advanced PSC (45%) than in mild disease (21%), suggesting that it promotes progression [52]. In MMP-3 gene encoding stromelysin, homozygosity for the 5A polymorphism was a significant risk for portal hypertension, indicative of a role in fibrogenesis [53].

Autoantibodies in PSC

Nuclear Envelope Autoantigens and Bacterial Mimicry

PSC is associated with a wide variety of autoAbs, many of which may be immunologic epiphenomena [64]. The most studied of the autoAbs in PSC are the atypical perinuclear antineutrophil cytoplasmic antibodies (pANCAs), which occur in ≤88% of PSC patients, with or without UC [55, 65, 66]. In PSC, IBD, and AIH, pANCA autoAbs rarely react with the classical pANCA Ags: cytoplasmic actin, catalase, or enolase [67]. Instead, the atypical pANCAs in PSC react with nuclear envelope Ags in neutrophils rather than cytoplasmic Ags. This changed their designation to peripheral antineutrophil nuclear antibodies (pANNAs) [67].

Analyses of pANNA epitope specificity showed that 92% of atypical pANNAs from patients with IBD or hepatobiliary diseases react with a 50 kDa myeloid-specific nuclear envelope protein [68] and subsequently identified a tubulin-beta isotype 5 [69]. Alpha and beta tubulins are highly conserved proteins that share 40% aa sequence homology, undergo multiple posttranslational modifications, and have multiple isotypes [70]. pANNAs against tubulin-beta isotype 5 were not PSC specific, but also occurred in AIH [69]. Subsequent studies showed that pANNAs react with the highly conserved bacterial cell division protein FTsZ and that preabsorption of PSC sera with FTsZ abolished pANNA reactivity. This indicates molecular mimicry between bacterial FTsZ and nuclear Ags of human neutrophils [71]. Of note, pANNA titers do not decrease after transplantation or colectomy for UC [67]. pANNAs also may be correlated with biliary complications [72], intrahepatic rather than extrahepatic strictures [73], and cirrhosis at high titers [74]. Unfortunately, these studies were not powered sufficiently to reach firm conclusions.

Future studies of circulating and liver-infiltrating CD4 and CD8 T cell TCR reactions against tubulin-beta isotype 5 with appropriate healthy and diseased controls should clarify the importance of this autoAg/bacterial molecular mimic in PSC pathogenesis. Computer modeling of the binding affinities of putative autoAg(s) for HLA class II molecules associated with PSC susceptibility and resistance may help define their Ag specificities.

IgG ANCA in the bile is correlated significantly with PSC risk and formation of dominant strictures, but not with risk of death, OLT, or cholangiocarcinoma [75]. The frequency of pANCA

is also significantly higher in UC than Crohn's disease [76]. Moreover, the combination of typical multi-Ag-specific ANCA, ANA, and SMA is 67 % sensitive for the diagnosis of PSC [76].

AutoAbs reacting with cholangiocytes have multiple consequences. The majority of PSC patients have serum IgA autoAbs that bind to cultured human cholangiocytes, while they are absent in the sera of healthy controls [33]. High titers correlated with total serum IgA levels and were clinically correlated with faster disease progression. IgG autoAbs in PSC sera also reacted against cultured human cholangiocytes and induced expression of TLR4 and TLR9 [77]. The addition of the LPS ligand for TLR4 and the CpG DNA ligand for TLR9 induced cholangiocytes to secrete copious amounts of proinflammatory cytokines, TNFα, Il-1β, and IL-6, along with IFNγ, TGFβ, and granulocyte-macrophage colony-stimulating factor. Bile ducts stained for TLR4 and TLR9 in biopsies of 58 % of PSC patients with IgG anti-cholangiocyte autoantibodies, indicating concordance between the in vitro observation and pathophysiology.

Induction of murine colitis by bacterial Ags and production of pANCA support the hypothesis that immune responses to bacterial Ags or other Ags cross-reactive with enteric Ags can induce pANNA in PSC [78, 79]. The fact that up to 81 % of PSC patients have antibodies against enterobacterial proteins also supports the hypothesis [65]. Bacterial/permeability-increasing protein (BPI), an endotoxin-binding neutrophil leukocyte-granular protein with antibacterial and antiendotoxin activity [80], is also an ANCA Ag in PSC, IBD, cystic fibrosis, and vasculitis [81]. Titers of BPI-ANCA correlate with inflammation and tissue damage, suggesting that BPI-ANCA might retard clearance of LPS, promoting inflammation and LPS stimulation of biliary TLR4 [77].

Cholangiocyte-Specific Autoantigens and CD44

Serum autoAbs reacting with human intrahepatic cholangiocytes from a healthy person were detected in 63 % of patients with PSC, 37 % with PBC, 16 % with AIH, and 8 % of healthy controls [82]. Western blotting showed that PSC patients exclusively had autoAbs reacting with a 40 kDa Ag. Anti-cholangiocyte antibodies from PSC and PBC patients, but not AIH patients, induced cholangiocyte secretion of proinflammatory IL-6, which stimulates cholangiocyte proliferation and inhibits apoptosis.

In PSC, but not PBC or AIH, both IgG and IgM autoAbs induced cholangiocyte expression of the CD44 cell adhesion receptor for the extracellular matrix ligand, hyaluronic acid, which also plays roles in cell proliferation, differentiation, presentation of cytokines, chemokines, and growth factors to their receptors, protease docking to cell membranes, and angiogenesis [83]. Blocking of the CD44v7 isoform on T cells and activated macrophages in an experimental murine model of IBD caused apoptosis of effector cells and clinical recovery [84]. Anti-CD44 reduced induction of experimental arthritis by collagen or proteoglycan PAMPs by preventing pathological interactions of synovial-like fibroblasts and cartilaginous matrix [85]. Thus, PSC-specific autoAbs against cholangiocyte autoAgs stimulate PSC-specific expression of CD44 isoforms potentially capable of reducing recruitment of effector leukocytes to the peribiliary space, suggesting the possibility of therapeutic inhibition of CD44 in PSC.

Nonspecific Autoantibodies

Multiple nonspecific autoAbs observed in PSC are likely epiphenomena related to chronic inflammation and immunogenetics favoring vigorous immune responses [64]. Frequencies of nonspecific autoAbs included antinuclear antibodies in 7–77 %, smooth muscle antibodies in 13–20 %, antimitochondrial antibodies in 0–9 %, anti-cardiologic antibodies in 4–66 %, anti-thyroperoxidase antibodies in 7–16 %, anti-thyroglobulin antibodies in 4 %, and anti-Ig rheumatoid factor in 15 %. AutoAbs against tropomycin found in either UC or PSC mediated antibody-dependent cellular cytotoxicity of cells expressing the HLA-DPw9 allele [86].

Immunological Epiphenomena

In addition to nonspecific autoAbs, multiple immunological abnormalities described in PSC

also appear to be epiphenomena consistent with the concept that PSC is associated with disordered immunoregulation [55, 64, 66]. These abnormalities include evidence of:(1) Decreased proportions of peripheral blood T cells and CD8 T cells [87, 88](2) Increased proportions of circulating B cells [89](3) Decreased T suppressor cell function [90](4) Increased autologous mixed lymphocyte reactivity [91](5) C' activation with increased levels of C3b and C4d [92](6) Deposits of C3d on hepatic arteries, but not bile ducts [93] (7) Immune complexes in the blood and bile [94] (8) Diminished clearance of artificial immune complexes by Kupffer cells in vivo [95] and (9) Aberrant expression of blood group antigens on biliary and colonic epithelia [96]

Cholangiocytes in the Immunopathogenesis of PSC

Cholangiocytes as Immunological Targets in PSC

Ductopenia occurs in PSC; however, inflammatory-mediated apoptosis of cholangiocytes is absent in PSC [13]. In contrast, apoptosis is the hallmark of CD8 T cell-mediated nonsuppurative destructive cholangitis (NSDC) leading to ductopenia in PBC, chronic graft-versus-host disease (GVHD), and hepatic allograft rejection (HAR) [11]. The near absence of NSDC in PSC [97] is intriguing, since PSC cholangiocytes express an activated phenotype of increased class I HLA, aberrant class II HLA, and ICAM-1 that would facilitate recognition by Ag-specific CD8 CTLs. Portal infiltrates in PSC also differ from those in PBC by containing neutrophils, CD4 T >>CD8 T cells, macrophages, NK, and γδT cells [44, 87, 88, 98, 99]. Evidence of a paucity of peribiliary CD8 CTLs in the precirrhotic stages of PSC strongly argues against cholangiocytes as primary target cells [88].

Immunomodulatory Roles of Cholangiocytes

It is now clear that cholangiocytes, rather than being passive target cells or innocent bystanders, play a seminal role in determining the composition of peribiliary inflammatory infiltrates and likely participate in periductular fibrogenesis in PSC (Fig. 9.3) [4, 8, 11, 100, 101]. Activated cholangiocytes express TLR4 and TLR9 for the PAMP ligands LPS and unmethylated CpG DNA molecules, respectively. Cholangiocytes also have receptors for proinflammatory cytokines TNFα, IL-1β, IL-6, as well as IFNγ. These stimuli induce cholangiocyte expression of chemokine receptors and secretion of multiple chemokines, cytokines, matrix metalloproteinases, and growth factors that immunomodulate inflammation and fibrogenesis (Fig. 9.3). Cholangiocyte secretion of multiple chemokines in PSC (Fig. 9.3) dictates the composition of peribiliary inflammatory infiltrates containing innate immune cells and T cells bearing specific chemokine receptors, including a pathogenetic population of PSC-specific T cells primed in the gut (discussed below) [8, 102]. Secretions of profibrotic TGFβ by activated cholangiocytes, along with profibrogenic cytokines secreted by peribiliary inflammatory cells, are likely causes of the concentric layers of circumferential fibrosis characteristic of PSC.

Endothelial Cells and the Role of Arterial Ischemia in PSC

Direct injury of hepatic arteries or arterioles causes secondary ischemic sclerosing cholangitis [103–105]. While there is no evidence of an immunological attack against endothelial cells of hepatic arteries or peribiliary capillary plexi in PSC [14, 97], it is now clear that concentric layers of circumferential peribiliary fibrosis progressively push peribiliary capillary plexi away from the basement membranes of bile ducts [14]. An experimental mouse model [106] suggests that a microcirculatory barrier to diffusion of O_2 and nutrients and disruption of the cholehepatic circulation created by fibrous displacement of the peribiliary capillary plexi might explain the atrophic, senescent appearance of cholangiocytes in PSC. An unsubstantiated but correlative hypothesis postulated that biliary ischemia resulted from aberrant production of angiotensin II or endothelin by PSC cholangiocytes, leading to vasoconstriction of peribiliary capillary plexi and arterioles [107].

Emerging Role of Gut Microbiota

Gut microbiota play essential roles in health and disease. Published studies are limited but indicate that gut microbial profile in PSC is distinctly different than that in UC without PSC or healthy controls [108]. Specifically, PSC patients have significantly reduced bacterial diversity compared with healthy controls and a different microbial composition compared to either controls or patients with UC alone. Microbiota were similar for PSC patients, regardless of the presence or absence of IBD. Eleven of 12 microbial genera were reduced in PSC, while the *Veillonella* genus (anaerobic, Gram-negative cocci) was significantly increased compared with controls of patients with UC. Of potential importance, the *Veillonella* genus is associated with other chronic inflammatory and fibrotic conditions. A study of ileocecal biopsies confirmed the low microbial diversity in the gut microbiota of PSC patients and noted significantly lower abundance of uncultured *Clostridiales II* compared with controls or patients with UC [109]. As noted above, FUT2 nonsecretors have low abundance of fecal bifidobacteria, *Proteobacteria*, and *Actinobacteria* and an increase in *Firmicutes* [37]. Finally, a study of the microbiota of the bile showed *Helicobacter pylori* DNA in microdissected hilar bile ducts in 9 of 56 (16%) end-stage PSC patients, suggesting that bile reflux can carry *H. pylori* into the distal biliary tract from the duodenum [110]. Further studies of the microbiota should lead to an understanding of the gut-liver axis in health and disease [111].

Immunopathogenic Role of Gut-Primed T Cells, Aberrant Expression of Adhesion Molecules, Chemokines, and Cytokines

A series of elegant studies have brought the immunomodulatory roles of cholangiocytes and the portal venous and arterial endothelia to the forefront of studies of PSC immunopathogenesis [8, 102]. Collectively, these studies demonstrated that hepatic inflammatory infiltrates in PSC contain T cells primed by Ags in gut-associated lymphoid tissues (GALT). These studies also link the immunopathogenesis of PSC to that of IBD [101, 112–116]. Early studies of extraintestinal manifestations of IBD in the eye, skin, and synovial tissues showed that inflammation was mediated by gut-primed lymphocytes that had inappropriately migrated to these tissues [9]. A similar pathogenetic mechanism in PSC did not appear likely, since PSC can occur in the absence of active gut inflammation, may be present years before the onset of IBD, or may even begin after total colectomy for UC. This led to the hypothesis that PSC is mediated by memory T cells primed in the gut that migrated into the peribiliary space as a result of aberrant expression of gut-specific adhesion molecules and cholangiocyte secretion of gut-specific chemokines [114, 116, 117]. Ag-specific activation of naïve T cells by gut DCs in Peyer's patches and mesenteric lymph nodes produces a gut-specific T cell phenotype (Fig. 9.3) characterized by expression of α4β7 and α4β1 integrins and chemokine receptors CCR9 and CCR10 [118, 119]. Hepatic DCs are incapable of imprinting this gut-specific phenotype.

Normally, circulating memory T cells of this phenotype interact only with gut endothelial cells, due to exclusive endothelial expression of the gut addressin mucosal vascular address cell adhesion molecule-1 (MADCAM-1) and the chemokine ligand CCL25, which bind to gut-primed T cell α4β7 and CCR9, respectively. Evidence that portal venous endothelial cells in PSC, but not other inflammatory liver diseases, aberrantly express MADCAM-1 and CCL25 provided a novel mechanism for the homing of gut-primed T cells into the portal tracts.

Further studies showed that the aberrant expression of MADCAM-1 on hepatic endothelial cells was caused by the physiologic interaction of natural dietary and microbial amines and vascular adhesion protein-1 (VAP-1) present on hepatic endothelial cells. VAP-1 functions as an adhesion molecule for the VAP-1 receptor (VAP-1R) and as an amine oxidase. The amine oxidase function of endothelial VAP-1 activates endothelial cell production of H_2O_2, which, in the presence of proinflammatory cytokines

(e.g., TNFα), leads to activation of NFkB and ultimately aberrant expression of MADCAM-1 and CCL25 by portal venous endothelial cells. In accord with VAP-1 roles in adhesion and amine metabolism, the absence of hepatic endothelial VAP-1 in VAP-1$^{-/-}$ knockout mice significantly reduced both portal inflammation and fibrosis in murine models of hepatic injury [8]. As discussed above, PSC cholangiocytes activated by cytokines, PAMPs, or autoAbs also secrete the CCL25 chemokine required for transendothelial migration of gut-primed T cells into the portal tracts (Fig. 9.3). Cholangiocyte secretion of CCL25 explains migration of gut-primed CCR9-positive T cells along the concentration gradient to the peribiliary space.

Peribiliary localization and survival of gut-primed T cells also involve cholangiocyte expression of additional adhesion molecules and chemokines [8]. Cholangiocyte expressions of CCL28 and vascular cell adhesion molecule-1 (VCAM-1) appear to play critical roles for peribiliary recruitment of gut-primed T cells expressing the α4β1 integrin receptor for VCAM-1 and the CCL28 ligand for the chemokine receptor CCR10. Since cholangiocyte expression of CCL28 has been observed in other chronic inflammatory liver diseases, its role in chemoattraction of CCR10-positive T cells is nonspecific. However, stimulation of cholangiocyte TLR4 with LPS and the proinflammatory cytokine IL-1β, both shown to be present in PSC, induces secretion of CCL28, augmenting the α4β1 interaction of T cells with cholangiocyte VCAM-1. In contrast, neither TNFα nor IFNγ induces cholangiocyte secretion of CCL28. Thus, the innate immune response of the cholangiocytes to LPS in a proinflammatory cytokine milieu appears necessary for transendothelial migration and peribiliary recruitment of gut-primed T cells.

Gut-primed T cells appear to be activated by enteric Ags or Ags that cross-react with enterocytes. T cell lines propagated from the inflamed common bile ducts of two PSC patients expressed oligoclonal TCRs, indicating recruitment of T cells activated by a limited number of Ags [120]. Since TCR oligoclonality was unchanged in a second biopsy performed more than a year later, it appeared that extrahepatic T cells expressing oligoclonal TCRs were repopulating the periductal tissue, possibly from mesenteric lymph nodes or Peyer's patches. These T cells proliferated in response to human enterocytes and mediated enterocyte cytotoxicity, consistent with gut-specific Ag stimulation. T cells from other PSC livers also preferentially expressed Vβ3 TCR [121], which did not correlate with the histopathological stage of disease.

Other studies showed that liver-infiltrating lymphocytes in PSC contain T cells that proliferate poorly to mitogens, have intracytoplasmic IL-1β and TNFα, and secrete copious amounts of IL-1β and TNFα and lower levels of IL-2, IL-10, and IFNγ in vitro [122]. Neither hepatic T cells nor NK cells were cytotoxic in vitro. Anti-TNFα antibodies partially restored the proliferation and cytotoxicity of PSC liver-infiltrating lymphocytes, suggesting an immunopathogenic role for high portal tract concentrations of TNFα. The fact that Kupffer cells in PSC are threefold greater in number than in other liver diseases [123] may increase the amounts of IL-1β and TNFα in peribiliary lymphatics. Serum levels of the major profibrotic cytokine TGFβ are also significantly increased in PSC, presumably due to secretion by Kupffer cells, portal macrophages, and cholangiocytes chronically stimulated by proinflammatory cytokines [123].

It remains unknown whether transendothelial migration of gut-primed T cells into the portal tracts can be mediated solely by hepatic endothelial cells expressing VAP-1 and aberrantly expressing MADCAM-1 and CCL25 or also requires expression of the original priming Ag(s). The absence of gut-primed T cell-mediated cytolysis of cholangiocytes suggests that cholangiocyte HLA molecules do not express priming antigenic peptides [13]. Chronic portal and peribiliary inflammation may be intensified by Th17 cells, and expression of multiple cholangiocyte adhesion molecules and chemokines induced by PAMPs and proinflammatory cytokines likely determines the composition of portal inflammatory infiltrates in PSC [34]. This may explain the fact that only 20 % of portal inflammatory cells

are gut-primed T cells [8]. However, the composition of the portal inflammatory infiltrates does not adequately explain why lesions of fibrous obliterative cholangitis associated with periductal concentric fibrosis occur only sporadically along the lengths of individual bile ducts and are absent in the small duct variant of PSC.

Key Unanswered Questions About PSC Immunopathogenesis

It remains unknown if circulating gut-primed, memory T cells provide immunological surveillance of both the liver and gut prior to initiation of PSC or only after hepatobiliary injury and proinflammatory cytokines facilitate VAP-1 induction of aberrant hepatic expression of MADCAM-1 and CCL25. Were livers of patients susceptible to PSC to express aberrant MADCAM-1 and CCL25 prior to the onset of PSC, it would suggest that the development of PSC requires a "second hit" such as cholangiocyte activation by PAMPs and proinflammatory IL-1β to induce VCAM-1 and secretion of CCL25 and CCL28 for recruitment and migration of gut-primed T cells to the peribiliary space.

Conversely, if VAP-1-mediated aberrant expression of hepatic endothelial MADCAM-1 and CCL25 were to occur only as an initial manifestation of overt PSC, then the etiopathogenesis of PSC would require a "multi-hit" hypothesis. Recurrence of PSC in transplanted liver allografts strongly suggests that aberrant expression of MADCAM-1 and CCL25 is not a primary expression of susceptibility but instead can be induced in a previously non-susceptible allograft. The role of the gut in posttransplant recurrence remains intriguing, since colectomy performed prior to or at the time of transplant protects against recurrence of PSC in UC patients. Colectomy performed later after transplant has no protective effect.

Animal studies support the key roles for PAMPs and proinflammatory cytokines in PSC immunopathogenesis [124]. PAMP-induced colitis with muramyl peptide [125] and *Escherichia coli* chemotactic peptide N-formyl L-leucine L-tyrosine (fMLT) [126] was complicated by PSC-like hepatic lesions. In genetically susceptible rats, the PAMP peptidoglycan-polysaccharide produced by small bowel bacterial overgrowth in a surgically created blind loop caused PAMP production, portal inflammation, bile ductular proliferation, and strictures of both intra- and extrahepatic bile ducts [127, 128]. Injury was correlated significantly with TNFα production by Kupffer cells. Mutanolysin cleavage of peptidoglycan-polysaccharide, palmitate blockade of Kupffer cell phagocytosis, and pentoxifylline inhibition of TNFα secretion by Kupffer cells prevented hepatobiliary inflammation and biliary strictures. These data are in accord with evidence that PSC susceptibility is associated with the class III HLA TNFA*2 allele and that patients with extended HLA-DR3 haplotypes secrete excessive amounts of TNFα. PAMPs and proinflammatory cytokines appear to play seminal roles in the immunopathogenesis of PSC.

Bile Regurgitation into the Peribiliary Space and Consequences of Biliary Obstruction

Bile contains noxious constituents, including toxic hydrophobic bile acids, PAMPs, and glycoproteins. Regurgitation of the bile into the peribiliary space as a result of disruption of the tight junctions between cholangiocytes results in toxic bile injury and periductal concentric fibrosis in the Mdr2 $(Abcb4)^{-/-}$ knockout mouse model [15]. Regurgitation of the bile into the peribiliary space induces neutrophilic inflammation, followed by lymphocytic infiltration and production of both proinflammatory cytokines and profibrotic TGFβ. As observed in PSC, progressive laminations of periductal fibrous tissue displace peribiliary capillary plexi, and cholangiocytes became atrophic, presumably due to microcirculatory ischemia and nutrient deprivation. Biliary casts showed focal, macroscopic strictures and ectasias similar to those seen in PSC. However, PSC is not associated with abnormal haplotypes for MDR3, the human homolog of murine Mdr2.

Although the bile in the Mdr2 $(Abcb4)^{-/-}$ knockout mouse contains increased proportions of hydrophobic bile acids, it is important to note that the bile also contains other constituents with potential roles in immunopathogenesis. For example, fMLT, a chemotactic peptide of *Escherichia coli* in portal venous blood, is also secreted by hepatocytes into the bile [129]. CD66a, also known as biliary glycoprotein, is also present in the human bile [130]. As the human homolog of rat cell adhesion molecule, it is expressed by neutrophils, monocytes, ductular epithelia, endothelial cells, gut intraepithelial lymphocytes, and myoepithelial cells within infiltrative scars and sclerosing adenosis of the breast [131]. CD66a binds to E-selectin, galectin-3, and bacterial type 1 fimbriae and CD66b/66c and inhibits the cytotoxic function of gut intraepithelial lymphocytes [132]. Thus, several constituents of the bile may modulate inflammation and possibly fibrogenesis if they were regurgitated into the portal tracts.

Contribution of Biliary Obstruction to Pathogenesis

Obstruction of the biliary tract results in increased proximal intraluminal pressures, increasing the potential for bile regurgitation. Experimental obstruction results in increased LPS concentrations in portal tracts; innate immune activation of Kupffer cells and portal tract macrophages by LPS and/or other PAMPs; secretion of proinflammatory cytokines IL-1β, TNFα, IL-6, TGFα/β, and leukotrienes by macrophages; leaky cholangiocyte tight junctions; and regurgitation of the bile into the peribiliary space [133, 134]. Accumulation of LPS inhibits cholangiocyte HCO_3^- secretion (required for the protective biliary bicarbonate umbrella) and compromises cholehepatic cycling between cholangiocytes and displaced peribiliary capillaries that may prevent removal of noxious molecules from the peribiliary space. A peribiliary milieu of proinflammatory cytokines, chemokines, and LPS recruits and activates neutrophils, monocytes, and T cells. Biliary obstruction also induces ductular proliferation of cholangiocytes lining canals of Hering at the margin of the portal tracts [97]. Proliferating ductules secrete platelet-derived growth factor (PDGF) [133], a potent mitogen for activated stellate cells, that promotes results in projections of fibrous septa into the parenchyma and, ultimately, secondary biliary cirrhosis.

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