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EDITORIAL

Autoantibodies in primary sclerosing cholangitis

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Abstract

The aetiology of primary sclerosing cholangitis (PSC) is not known and controversy exists as to whether PSC should be denominated an autoimmune disease. A large number of autoantibodies have been detected in PSC patients, but the specificity of these antibodies is generally low, and the frequencies vary largely between different studies. The presence of autoantibodies in PSC may be the result of a nonspecific dysregulation of the immune system, but the literature in PSC points to the possible presence of specific antibody targets in the biliary epithelium and in neutrophil granulocytes. The present review aims to give an overview of the studies of autoantibodies in PSC, with a particular emphasis on the prevalence, clinical relevance and possible pathogenetic importance of each individual marker.

Key words: Primary sclerosing cholangitis; Autoantibodies; Autoimmunity; Antibodies against cytoplasmic constituents of neutrophil; Tropomyosin

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of the intra- and extrahepatic biliary tree leading to progressive bile duct strictures and liver cirrhosis[1]. No effective medical treatment is currently available[2] and PSC is a major indication for liver transplantation[3]. The PSC population is heterogeneous, comprising subgroups of regular "large-duct" PSC, patients with "small-duct" affection only[4] and an "overlap-syndrome" between PSC and autoimmune hepatitis (AIH)[5]. Up to 80% of the PSC patients have concurrent inflammatory bowel disease (IBD)[6]. According to standard endoscopic and histological criteria, the IBD is most often classified as ulcerative colitis (UC), but there is also an association with colonic Crohn's disease (CD)[7,8].

The aetiology of PSC is unknown (Figure 1). Immune responses against self antigens in the bile ducts have been proposed to play an important role in the pathogenesis, although controversy exists as to whether PSC should be denominated an autoimmune or merely immune mediated disease[9]. On one side, there are several lines of evidence supporting classification of PSC as an autoimmune disease[10]. This evidence includes (1) association with other autoimmune diseases in the same individual[11] and first degree relatives[12], (2) infiltration of T-lymphocytes in the portal tracts[13] with restriction in T cell receptor V gene usage[14], (3) a statistical association with particular human leukocyte antigen (HLA) haplotypes[15] and (4) the presence of autoantibodies[16]. On the other side, there is no documented effect of immunosuppressants in PSC[2], and in contrast to the female predominance of many diseases regarded as autoimmune, approximately 2/3 of PSC patients are male[17]. These notions suggest that additional pathogenetic factors may exist (e.g. bile acid toxicity[18]), and to what extent and at what disease stage autoimmune mechanisms contribute to the bile duct damage observed in PSC is not known.

In many autoimmune diseases, autoantibodies serve as markers of disease activity, may aid in the diagnosis of patients, and provide important insight into the pathogenesis. In clinical practice, a good

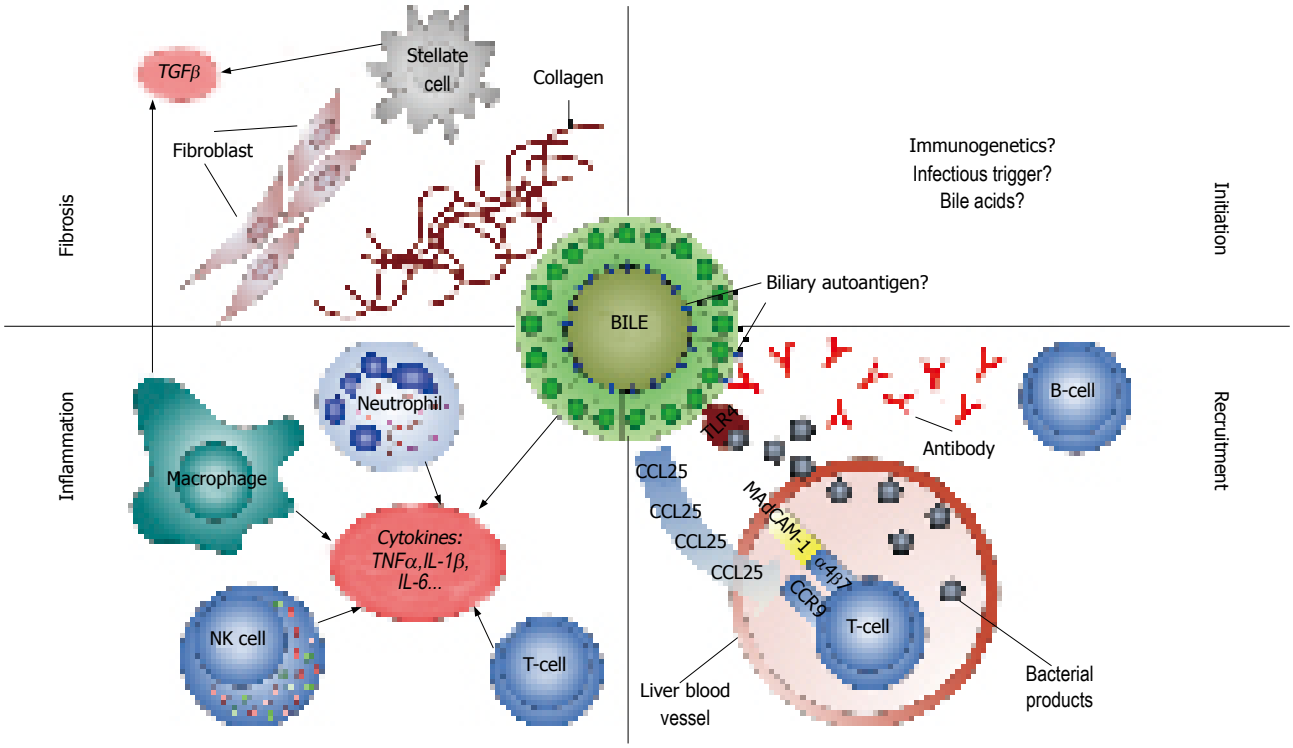

Figure 1 Schematic illustration of key elements of PSC pathogenesis. Initiation (upper right): The initiating factor(s) of PSC pathogenesis are unknown. Immunogenetic factors (including the presentation of autoantigens on PSC associated HLA molecules), an infectious trigger, and toxic or immunological effects from bile acids have been proposed. Recruitment (lower right): Autoantibodies produced by B-lymphocytes bind to biliary epithelial cells (BECs), leading to inflammation when there is concomitant stimulation of toll like receptors (TLRs) by bacterial products [LPS and other pathogen-associated molecular patterns (PAMPs)] from the gut. Recruitment of gut-primed (α4β7) T-lymphocytes in inflammatory bowel disease may contribute to the inflammation because of aberrant expression of the MadCAM-1 ligand in the endothelium and production of the CCL25 chemokine by BECs. Inflammation (lower left): T-lymphocytes and natural killer (NK) cells predominate in PSC affected livers, but neutrophils and macrophages are also recruited. Together with activated BECs they are sources of the cytokines and chemokines that perpetuate the inflammation in PSC. A specific cellular component of neutrophils (tubulin beta 5 chain), has been hypothesized to serve as an autoantigen in this inflammatory process, possibly cross-reacting with the bacterial homolog FtsZ and leading to the generation of anti-neutrophil cytoplasmic antibodies (ANCAs). Fibrosis (upper left): Characteristically in PSC, there is extensive fibrosis and stricturing of the bile ducts. Resulting from the inflammation and possibly concomitant bile leakage, pro-fibrotic factors [e.g. transforming growth factor beta (TGF-β)] from macrophages and/or stellate cells are ultimately responsible for the fibrotic obliteration of the bile ducts and liver cirrhosis in PSC.

marker is sensitive and specific and yields prognostic information [e.g. anti-cyclic citrullinated proteins (anti-CCP) antibodies in rheumatoid arthritis (RA)]. In studies of pathogenetic mechanisms, a good marker is tissue specific and closely linked to other observations regarding the pathogenesis (e.g. TSH receptor antibodies in Graves' disease). In PSC patients, a large number of different autoantibodies have been reported (Table 1). Some of these autoantibodies react with biliary or colonic epithelial antigens, others with constituents of neutrophil granulocytes, and some even with various ubiquitously expressed self antigens.

One of the most consistent findings regarding the aetiology of PSC is the disease association with genetic variants within the HLA-complex on chromosome 6[15]. HLA class I and II genes encode molecules which present antigens to $CD8^+$ and $CD4^+$ T-lymphocytes, respectively, resulting in an immune response against the antigen when appropriate co-stimulation is present[19]. A relationship between particular autoantibodies and disease associated HLA variants has been detected in other autoimmune diseases[20], but in PSC the pathogenetic importance of most of the identified autoantibodies is poorly defined. The present editorial aims to give an overview of the studies of autoantibodies in PSC, with a particular emphasis on the prevalence, clinical relevance and possible pathogenetic importance of each individual marker.

ANTIBODIES AGAINST BILIARY AND COLONIC EPITHELIAL ANTIGENS

The identification of antibodies against well defined biliary antigens in PSC would strongly support the hypothesis of an autoimmune aetiology. Given the high frequency of colitis among the patients, such antigens could potentially also be expressed in the colonic mucosa.

One autoantibody of this type was proposed by Das *et al*[21], who identified an antigenic protein expressed in both colonic and biliary epithelium, in addition to eye, skin and cartilage[22]. This 40 kDa protein was identified as human tropomyosin isoform 5 (hTM5)[23-25]. A monoclonal antibody (Das-1) was developed, and serum from UC and PSC patients inhibited the binding of Das-1 to the epithelium, indicating antibodies

Table 1 Autoantibodies detected in PSC patients

Antibody	Prevalence (%)	(Median)	No. of patients	(Median)	No. of articles
Anti-BEC	63	(63)	30	(30)	1[36]
pANCA	26-94	(68)	13-86	(30)	19 (Table 2)
AMA	0-9	(0)	15-73	(37)	10[44,61,78,89,102,112,137-140]
Anti-LKM	0	(0)	10-80	(37)	7[44,89,112,137,140-142]
Anti-SLA/LP	0	(0)	10-37	(25)	4[44,89,140,142]
ANA[1]	8-77	(30)	13-73	(35)	13[44,61,78,89,99,101,102,111,112,137-140]
SMA[1]	0-83	(17)	10-73	(36)	10[44,61,78,89,111,112,137-140]
ASCA	44	(44)	25	(25)	1[115]
Anti-cardiolipin	4-63	(27)	23-73	(41)	3[61,78,87]
Rheumatoid factor	15	(15)	71	(71)	1[78]
AECA	35	(35)	20	(20)	1[87]
Anti-TPO	16	(16)	73	(73)	1[78]
Anti-GBM	17	(17)	24	(24)	1[87]
Anti-sulfite oxidase	33	(33)	39	(39)	1[130]
Anti-GSTT1	5	(5)	58	(58)	1[133]

PSC: Primary sclerosing cholangitis; Anti-BEC: Antibodies against biliary epithelial cells (measured with flow cytometry); PANCA: Perinuclear antineutrophil cytoplasmic antibodies; ANA: Antinuclear antibodies; SMA: Smooth muscle antibodies; ASCA: Anti saccharomyces cerevisiae antibodies; AMA: Anti-mitochondrial antibodies; Anti-LKM: Liver-kidney microsomal antibodies; Anti-SLA/LP: Antibodies against soluble liver antigen/liver pancreas; AECA: Anti-endothelial cell antibodies; Anti-TPO: Antibodies against thyroid peroxidase; Anti-GBM: Antibodies against the glomerular basement membrane; Anti-GSTT1: Antibodies against glutathione S transferase theta 1. [1]In the largest cohort investigated, ANAs and/or SMAs were detected in 22% (24/111) of the PSC patients, but the frequency of each type was not given[143].

against hTM5 related epitope(-s) in the sera[22,26]. In the cell membrane hTM5 is found complexed with a 200 kDa colonic epithelial protein (CEP), and this complex is speculated to serve as the true target for the Das-1 antibody[27]. Antibodies against hTM5 have been detected in UC patients without PSC[28], and anti-hTM5 in UC sera has recently been shown to induce cytotoxicity against colonic epithelial cells *in vitro*[29]. In PSC patients without concomitant UC, a single study identified antibodies against a 9-amino acid sequence from hTM (not isoform specific) in 100% (8/8) of patients as compared with 69% (33/48) of UC patients and 0/6 PBC patients[30]. The findings of Das *et al* have been partly reproduced by others[31], but given a number of critical concerns[32-35], further studies are required to conclusively confirm and elaborate the importance of the hTM5-CEP antigen in the pathogenesis of PSC.

A Swedish group has reported on the presence of antibodies against isolated biliary epithelial cells (BEC) at high frequencies in sera from PSC (63%) and PBC (37%) patients, *versus* 8% of controls (1/12)[36]. A 40 kDa antigenic protein was identified, but this protein did not react with tropomyosin antibodies, which implies that either the 40 kDa protein in this study is not a tropomyosin isoform or the antibody used reacts with other tropomyosin isoforms. Anti-BEC from PSC sera (and to a lesser extent PBC sera) induced isolated BEC to produce IL-6 and the adhesion molecule CD44, strongly suggesting pathogenetic importance. Recently the group also showed that sera from PSC patients with anti-BEC stimulated BEC to express toll-like receptors (TLR), leading to BEC cytokine production upon exposure to lipopolysaccharide (LPS, endotoxin) from gram negative bacteria[37]. This means that both LPS and antibodies against BEC are necessary to activate BEC and generate cytokine release. An association between the presence of the anti-BEC and PSC associated HLA haplotypes (DR2 and DR3) was also suggested. The relevance of the Swedish findings are further strengthened by a higher frequency of acute liver transplant rejection in patients with anti-BEC prior to transplantation (all liver diseases) than in patients with no anti-BEC[38]. However, it needs to be noted that in this study there was a high prevalence of anti-BEC in all end stage liver patients (HCV 32%, PSC 56%, PBC 75%, HBV 57%, AIH 57%, and alcoholic cirrhosis 71%). This raises concerns as to the PSC specificity of the antibody, which clearly needs to be characterised prior to further studies.

Taken together, the findings of Das *et al* and the Swedish group suggest that antigens expressed in the biliary epithelium may induce self-reactive immune responses under certain conditions. Whether the antigenic epitope(s) lie within the hTM5-CEP complex or elsewhere remains to be elucidated, and the clinical significance of the corresponding autoantibodies must be established.

ANTIBODIES AGAINST NEUTROPHILS

Antibodies against cytoplasmic constituents of neutrophils (ANCAs) were initially described in patients with glomerulonephritis and systemic vasculitis[39,40]. In UC patients, antibodies against nuclear antigens were reported by Calabresi *et al* in 1961[41] and Nielsen *et al* in 1983 (granulocyte specific-ANA)[42]. In PSC such antibodies were reported by Snook *et al* in 1989[43]. These antibodies are also present in a large proportion of patients with AIH[44] and the name ANCA was applied due to the close resemblance to ANCAs found in several of the vasculitides[45,46]. ANCA is analyzed by incubating fixated human neutrophil slides with patient serum, and subsequently with secondary antibodies conjugated to a fluorophore. The indirect immunofluorescence

Table 2 Prevalence of pANCA[1] in PSC patients and controls[2] [% ($n^{\text{antibody positive}}/n^{\text{total population}}$)]

Authors	PSC	PSC -IBD	PSC+IBD	UC -PSC	CD-PSC	AIH	PBC	HC	MT
Terjung *et al*[44]	94[3]					81[4] (142/175)	31	0	IIF
	(33/35)						(14/45)	(0/19)	1:10
Klein *et al*[54]	87			78	27			0	IIF
	(26/30)			(18/23)	(16/60)			(0/20)	1:10
Mulder *et al*[102]	79	77[5]	82[5]			88[6]	28	5	IIF
	(19/24)	(10/13)	(9/11)			(21/24)	(7/25)	(12/252)	1:32
Lo *et al*[56]	77[7]			33	0	33[6]	0	0	AP
	(23/30)			(15/45)	(0/32)	(1/33)	(0/14)	(0/50)	1:10
Seibold *et al*[98]	775	40[8]	88	83	25	33[6]	28	0	IIF
	(17/22)	(2/5)	(15/17)	(38/46)	(20/80)	(5/15)	(7/28)	(0/30)	1:10
Gur *et al*[87]	75	75	75[5]						IIF
	(15/20)	(3/4)	(12/16)						1:20
Muratori *et al*[144]	75					31[4]	2	0	IIF
	(18/24)					(12/39)	(1/51)	(0/18)	1:20
Seibold *et al*[84]	72	50	76[5]	62	4	35[4]	28	0	IIF
	(18/25)	(2/4)	(16/21)	(30/48)	(2/48)	(8/23)	(6/21)	(0/40)	1:10
Zauli *et al*[137]	72								IIF
	(33/46)								-[9]
Hardarson *et al* [145]	69	75	67[5]	76	8	50[6]	0		IIF
	(20/29)	(6/8)	(14/21)	(16/21)	(2/25)	(10/20)	(0/33)		1:40
Roozendaal *et al*[99]	67								IIF
	(46/69)								1:40
Bansi *et al*[146]	66[10]					65[6]	13	0	AP
	(57/86)					(11/17)	(7/55)	(0/36)	1:5
	51[10]					65[6]	11	0	IIF
	(44/86)					(11/17)	(6/55)	(0/36)	1:5
Bansi *et al*[101]	65	29	70[5]	45				0	AP
	(41/63)	(2/7)	(39/56)	(38/85)				(0/36)	1:05
Tervaert *et al*[88]	62					71[6]	33	0	IIF
	(8/13)					(5/7)	(5/15)	(0/24)	-[9]
Roozendal *et al*[57]	49					70[11]	15	0	IIF
	(27/55)					(62/88)	(8/53)	(0/78)	1:40
Claise *et al*[53]	44	25	60	37	15	24[11]	0	0	IIF
	(12/27)	(3/12)	(9/15)	(18/49)	(11/75)	(25/105)	(0/30)	(0/50)	1:20
Vermeulen *et al*[147]	44			56	15	46[6]		5	IIF
	(16/36)			(56/100)	(15/100)	(17/37)		(5/105)	1:40
Wilschanski *et al*[60]	29								IIF
	(7/24)								1:20
Pokorny *et al*[100]	26	29	23			22[6]	0		IIF
	(10/39)	(5/17)	(5/22)			(2/9)	(0/7)		1:20

PSC: Primary sclerosing cholangitis; IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; HC: Healthy controls; MT: Method and titre considered positive (cut-off); IIF: Indirect immunofluorescence; AP: Alkaline phosphatase method. [1]No distinction between classical/atypical; [2]The single largest study of autoantibodies in PSC reported ANCAs among 84% (61/73) of the patients, but this study did not apply IIF, meaning that this figure is the total of patients with any ANCA subspecificity[78]; [3]Atypical pANCA (as opposed to classical pANCA or cANCA); [4]autoimmune hepatitis type 1 and 2; [5]Calculated from article values; [6]Autoimmune hepatitis not subclassified (1 or 2); [7]ANCA "type 1 pattern" is interpreted as pANCA, including both IgA/IgM/IgG; [8]Significant difference between PSC +IBD and PSC -IBD (*P* value not given); [9]Details not given, correspondence, not peer-reviewed; [10]Calculated sum of 4 patient populations from different countries; [11]Autoimmune hepatitis type 1.

(IIF) pattern is classified as cytoplasmic (cANCA) or perinuclear (pANCA)[47,48]. Billing *et al*[49] and Terjung *et al*[50-52] have made an additional contribution to this nomenclature, documenting that the main ANCA pattern in PSC, AIH and UC is "atypical". This means that the likely antigen is located in the nucleus rather than in the cytoplasm. The names anti-neutrophil nuclear antibodies (ANNAs)[51] and nuclear anti-neutrophil antibodies (NANAs) have thus been proposed[49].

The prevalence of ANCA (subtype not specified) in PSC patients ranges from 42% to 93%[45,53-61], and that of the pANCA subtype from 26% to 94% (Table 2). Comparable prevalences of ANCA are reported in AIH and UC (Table 2). No definite evidence links ANCA to the genetic susceptibility of PSC in terms of particular HLA haplotypes[62]. One study has reported on an increased prevalence of ANCA in PSC relatives as compared with healthy controls[63] while another study could not confirm this[64].

Nuclear specificities of the neutrophil antigens

Multiple neutrophil antigens contribute to different ANCA IIF patterns (Table 3). A study published in abstract form by Terjung *et al*[65] in 2005 proposed that the main antigen of atypical pANCA in AIH, UC and PSC patients is tubulin beta 5 chain (TBB5), a nuclear membrane-associated protein present in myeloid cell lines. Further studies of anti-TBB5 are necessary to characterise the clinical and pathogenetic relevance of these findings. Other nuclear antigens have also been

Table 3 Prevalence of antibodies against a selection of specific neutrophil antigens in PSC patients

Antibody	Frequency range % (median)	No. of patients range (median)	Number of studies
Anti-lactoferrin	4-50 (29)	12-76 (24)	10[55,57,84,85,87,88,99,102,137,144]
Anti-myeloperoxidase	0-33 (2)	12-73 (40)	7[57,78,84,85,87,99,102]
Anti-BPI	5-46 (29)	36-76 (69)	5[55,57, 59,78,99]
Anti-cathepsin G	0-35 (21)	14-76 (55)	5[55,57,84,87,99]
Anti-proteinase 3	0-44 (4)	25-73 (62)	5[57,78,87,99,102]
Anti-elastase	0-35 (9)	23-76 (69)	4[55,87,99,102]
Anti-α-enolase	11-33 (27)	15-55 (36)	3[57,89,147]
Anti-catalase	16-60 (38)	15-55 (35)	2[57,89]
Anti-α-antigen	33 (33)	12 (12)	1[85]
Anti-h-lamp-2	71 (71)	73 (73)	1[78]
Anti-TBB5[1]			

PSC: Primary sclerosing cholangitis; Anti-BPI: Antibodies against bactericidal/permeability increasing protein; Anti-h-lamp-2: Antibodies against human lysosomal-associated membrane protein 2; Anti-TBB5: Antibodies against Tubulin beta-5 chain. [1]No prevalence studies published.

proposed as nuclear targets of pANCA in AIH and UC, notably the high mobility group (HMG) non-histone chromosomal proteins HMG1 and HMG2[66-68] and Histone H1[69]. These have not been studied in patients with PSC.

Cytoplasmic specificities of the neutrophil antigens

A variety of cytoplasmic proteins have also been proposed to be targets for ANCAs in PSC. In ANCA-associated small vessel vasculitis (Wegener's disease, microscopic polyangiitis and Churg-Strauss syndrome) the main proportion of specific ANCAs are directed against proteinase 3 (PR3, mainly cytoplasmic IIF pattern) and myeloperoxidase (MPO, mainly perinuclear IIF pattern)[70]. In these diseases, increased ANCA levels may predict clinical relapse, but there is limited correlation between titres and disease activity. The prevalence of anti-PR3 and anti-MPO in PSC patients is low (Table 3).

Bactericidal/permeability increasing protein (BPI) has functional domains which bind the inner core region of LPS[71]. This binding triggers anti-bacterial activity, neutralization of endotoxin and delivery of endotoxin rich particles to host cells[72]. Anti-BPI is detected in many clinical settings. In PSC, anti-BPI has been found in 5% to 46% of the patients (Table 3), which is similar to UC (3%-39%)[59,73-77], compared with 0% to 5% of healthy controls[57,78]. Anti-BPI is also reported in RA, systemic lupus erythematosus (SLE) and systemic sclerosis[79], and interestingly there is a high prevalence of anti-BPI in cystic fibrosis patients colonized with gram negative bacteria[80].

Another LPS-binding ANCA target is lactoferrin, which is released from neutrophils during inflammation and has bactericidal and immune modulating effects[81]. Antibodies against lactoferrin have been detected in several autoimmune diseases including RA[82], SLE[83], reactive arthritis[82] and ankylosing spondylitis. The reported prevalence of anti-lactoferrin in PSC (4%-54%, Table 3) is similar to that in UC (4%-50%), and considerably higher than in CD (0%-9%)[73,75,76,84-86] and healthy controls (0%)[87,88].

Antibodies against the proteases elastase and cathepsin G are found in up to 35% of patients with PSC (Table 3). Catalase prevents cell damage from reactive oxygen-derived free radicals, and antibodies against catalase have been detected in up to 60% of PSC patients, compared with up to 10% of healthy controls[57,89]. Finally, human lysosomal-associated membrane protein 2 (h-lamp-2) is a target of ANCA in vasculitides[90]. In a single study, anti-h-lamp-2 was detected in a large proportion of PSC patients (71%) *versus* only 15% of healthy controls[78]. No disease controls were investigated. This finding has not yet been reproduced.

Pathogenetic role of ANCAs

The large range of different ANCAs in PSC (Table 3) has been critically interpreted as the ANCAs serving as nonspecific epiphenomena of an immune response against dying neutrophils at an inflammatory site[91,92]. ANCAs (i.e. anti-MPO and anti-PR3) may, however, activate neutrophils[70], and anti-BPI may inhibit clearance of LPS[93]. Also, widely and even ubiquitously expressed antigens sometimes serve as antigens in tissue specific autoimmunity [e.g. anti-mitochondrial antibodies (AMAs) in PBC].

Another possibility is related to the predominant theory on UC and CD, which involves an aberrant response to gut luminal antigens in genetically susceptible hosts[94]. A series of antibodies against bacterial antigens have been detected in IBD patients, and ANCAs may represent such antibodies[94]. One study from 1995 indicated that colonic lamina propria B-cells in UC produce pANCA[95]. In another study, absorption of human pANCA-positive sera with enteric bacterial antigens reduced or abolished the specific perinuclear staining[96]. The targets of these pANCAs are not known, but a study published in abstract form in 2006 indicates that antibodies giving rise to the atypical pANCA pattern have dual reactivity against both TBB5 and the microbial tubulin FtsZ[97]. How these cross-reacting antibodies may lead to hepatobiliary pathology can only be speculated upon.

Diagnostic and clinical relevance of ANCAs

The sensitivity of ANCA in PSC is high in some studies,

whereas specificity is low. In one study of the diagnostic precision of autoantibodies in liver diseases, atypical pANCA with cut-off titre 1:40 had a specificity of 78% and sensitivity of 61% for PSC (AUC, 0.69; 95%CI, 0.61-0.77)[44]. Identification of the principal antigenic target of ANCAs in PSC would allow prospective studies to define this diagnostic role further. Currently, ANCA does not contribute diagnostically or during the clinical follow-up of PSC patients.

In terms of correlation between ANCA and particular clinical characteristics of PSC, no clear interpretation can be made from available data. If ANCAs were to represent markers for intestinal affection in PSC, a higher ANCA prevalence should be detected in PSC patients with IBD than in patients without IBD. This has only been shown in one small study by Seibold *et al*[98]. In another study, anti-lactoferrin was more prevalent in PSC with UC than without[99]. A few papers relate ANCA positivity to biliary tract complications like biliary calculi or cholangiocarcinoma[100], or more extensive involvement of the biliary tree (both intra- and extrahepatic as compared with intrahepatic only)[101]. The presence of pANCA has also been found to correlate with disease stage (cirrhosis or liver transplantation)[100,102], and in one study anti-BPI and anti-cathepsin G were more prevalent in PSC patients with cirrhosis[99]. Most other papers reported no difference in ANCA positivity between early and advanced PSC, and found no correlation between titres and disease activity[45,57,99,103]. ANCAs seem to persist after liver transplantation[98,104], even though the titres may vary during follow-up[103].

AUTOANTIBODIES SPECIFIC TO LIVER DISEASES OTHER THAN PSC

AMA may be considered one of the most useful autoantibodies in the diagnosis of cholestatic liver disease, since AMAs are virtually absent in PSC patients (Table 1) compared with a 90%-95% prevalence in PBC[105]. The AMA antigens are different epitopes of the pyruvate dehydrogenase complex (PDC), especially the PDC-E2[106,107]. Mitochondrial antigens are expressed in all nucleated cells, and AMAs are classically detected by IIF. The presence of AMAs in PBC is an example of how autoimmunity against a ubiquitous antigen may be involved in the pathogenesis of a highly tissue specific disease. One of several proposed theories in PBC hypothesizes that in biliary epithelial cells the main AMA-antigen (PDC-E2) is not glutathiolated (as opposed to in other cells), causing persisting antigenicity of PDC-E2 when biliary epithelial cells undergo apoptosis[108]. Modification of AMA antigens in the liver by xenobiotics may also contribute[108]. A similar post-translational modification of proteins is known to contribute to antigenicity in several autoimmune diseases (e.g. antibodies against citrullinated proteins in RA)[109].

Anti-liver kidney microsomes type 1 (anti-LKM1), anti-soluble liver antigen/liver pancreas antigen (anti-SLA/LP) and anti-liver cytosolic protein type 1 (anti-LC1) are autoantibodies used in diagnosis of AIH[105]. These have not been detected in PSC patients (Table 1).

ANTINUCLEAR (ANAS) AND SMOOTH MUSCLE ANTIBODIES (SMAS)

ANA and SMA are directed against ubiquitous antigens. ANA is the hallmark of SLE and other connective tissue diseases, but are also among the most prevalent autoantibodies in AIH[110]. ANAs may represent a large number of nuclear targets while SMAs are similarly undefined and directed against actin and other cytoplasmic filamentous proteins. ANA is reported in 8%-77% of the PSC patients (Table 1). No particular ANA subspecificities seem to predominate; anti-dsDNA has been reported in 3%-29%[61,78,87,111], anti-ENA in 4%-12%[78,111,112], anti SSA/B in 1%-28%[78,87] and anti-RNP, anti-SCL70, anti-Sm and anti-ssDNA in a minority of patients[87]. SMAs have been reported in 0%-83% of PSC patients (Table 1) but the prevalence is also high in AIH, various malignancies and infections[107]. ANA and SMA often co-exist, they lack organ and disease specificity, and should probably be concluded as irrelevant for the diagnostic process and pathogenesis in PSC.

ANTIBODY AGAINST SACCHAROMYCES CEREVISIAE (ASCA)

ASCA is an antibody against baker's yeast (microbial antigens) and therefore does not represent a typical autoantibody. ASCA was first described in patients with CD in 1988[113]. The antigenic epitope of ASCA is located on the S. cerevisiae mannan, which is a polymer of mannose[114]. In a single study, 44% (11/25) of PSC patients were ASCA positive (57% with concurrent IBD and 39% without IBD) compared with 23% (28/123) of PBC and 18% (12/67) of AIH patients[115]. The presence of ASCA is interesting as a specific example of immune responses towards gut luminal antigens in IBD. As a serological marker in PSC, however, ASCA does not seem to contribute.

ANTI-PHOSPHOLIPID ANTIBODIES

Anti-phospholipid antibodies are directed against phospholipids or phospholipid associated proteins, and are associated with thromboembolic disease. They are commonly detected in connective tissue disorders (e.g. SLE) but also in 1% to 5% of healthy subjects and during infections[116]. Three studies have investigated the presence of anti-cardiolipin antibodies in PSC with the prevalence ranging from 4% to 63% (Table 1). Interestingly, Angulo *et al*[78] found a positive correlation between anti-cardiolipin titres and Mayo risk score and histological disease stage, and there are anecdotal reports of an elevated risk of thrombosis in PSC patients[117]. An increased risk of hepatic artery thrombosis post liver transplantation has also been proposed[118]. Anti-

cardiolipin antibodies have also been reported at low frequencies in UC (16%-26%)[119-121] and CD (16%-27%) patients[120,121].

OTHER AUTOANTIBODIES

In addition to ANA and SMA, several other non-specific autoantibodies are detected in PSC. Rheumatoid factor is detected in connective tissue diseases, infections and lymphoproliferative diseases[122], but is only found in 15% of PSC patients[78]. Anti-endothelial cell antibodies (AECAs) are directed against antigens in endothelial cells and have been reported in 35% of PSC patients in a single small study[87] but are observed in many other clinical conditions including vasculitis, SLE, systemic sclerosis and IBD[123,124]. The clinical and pathogenetic roles of AECAs are not clear[123,124].

A few autoantibodies in PSC are probably related to co-morbidity. In one study, the prevalence of thyroid diseases in PSC patients was 8%[11]. This probably explains the elevated levels of anti-thyroid peroxidase (Table 3) and other thyroid related antibodies in PSC patients[54,78,87]. An association between PSC and celiac disease has been reported[125-127]. Recently the celiac disease related anti-tissue transglutaminase was detected in 7% of PSC patients in a large pre-transplant cohort from the Mayo Clinic (11/155), *versus* 6% (7/112) of PBC and 35% (15/43) of AIH[128] patients. This may in part be explained by shared susceptibility HLA-alleles (DQ2 and DQ8)[129]. Finally, in a single small study, antibodies against the glomerular basement membrane (anti-GBM) were detected in 17% of patients with PSC, while all healthy controls were negative[87]. The significance of this finding is not known.

Antibodies against sulfite oxidase were detected by Preuss *et al*. in 33% (13/39) of PSC patients compared with 5% (5/96) of PBC and 9% (7/77) of AIH patients[130]. Sulfite oxidase is a mitochondrial enzyme previously thought to be the antigen of anti-M4 (an AMA subtype)[131] but this does not seem to be correct[132]. The authors report lower prevalence in PSC patients treated with UDCA but the role of anti-sulfite oxidase antibodies in PSC remains to be established[130].

Glutathione S-transferase theta 1 (GSTT1) was recently investigated as a candidate autoantigen in PSC by Ardesjö *et al* using immunoscreening[133]. This group created a cDNA library based on mRNA from human ductus choledochus[133]. The GSTT1 antigen was identified screening one single PSC patient serum for antibodies against bacteria expressing the cDNA encoded proteins. Upon testing in a larger population of PSC patients (n = 58), antibodies against GSTT1 were only found in three patients, thus concluding GSTT1 as unlikely to serve as an important autoantigen in PSC. Nevertheless, the study points to the possible need for the application of broader screening methods in the search for autoantigens in PSC. The role of autoantibodies and B-cells in other autoimmune diseases has gained renewed interest the last few years[134], not only as pathogenetic factors[135], but also as therapeutic targets (e.g. Rituximab)[136]. It is thus likely that further insight into the role of autoantibodies in PSC may be of clinical importance and further studies are warranted.

CONCLUSION

A large number of autoantibodies have been detected in PSC patients. The specificity of these antibodies is generally low and the frequencies vary largely between different studies. Interpretation of the literature is difficult because of small patient sample sizes and variable methodology for antibody detection. The presence of autoantibodies in PSC is often attributed to a nonspecific dysregulation of the immune system, but the literature in PSC points to the possible presence of specific antibody targets both in the biliary epithelium and in neutrophils. Further characterisation of such targets would probably yield important insight into the pathogenesis of PSC. The investigation of larger populations may also further define the role of autoantibodies in PSC as diagnostic tools.

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