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Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing cholangitis

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Abstract Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic autoimmune-mediated diseases of the biliary tree, resulting in a loss of bile ducts. There are morphological features that clearly distinguish them from each other: in PBC, there is overt destruction of the bile ducts with disruption of the basement membrane; in PSC there is abundant periductular fibrosis with shrinkage and subsequent loss of the bile ducts. In order to see if the disparate histopathology is paralleled by different immunohistology we looked at a panel of epitopes on bile duct epithelia especially to see if biliary epithelial cells may present as targets for cell mediated immune respone. In PBC bile duct epithelial cells mostly expressed CD58 (lymphocyte function-associated antigen 3), CD80 (B7 BB1), and CD95 (Fas). In PSC, however, these epitopes were only expressed in a few examples to a lower degree. The respective effector T lymphocytes were positive for CD2 and CD28. Subtyping of the lymphocytes in the liver tissue further showed a predominance of CD4 positive T cells over CD8 cells up to 2-to-1 in both diseases. Determination of lymphocytes by cytokines to Th1 or Th2 subtype showed a majority of Th1 lymphocytes in PBC and PSC. We conclude that in PBC bile duct epithelial cells may display features of target cells of a T cell-mediated immune reaction with the Th1 cells predominating. In PSC other mechanisms of bile duct loss may play a role, since in this disease the majority of cells lack essential epitopes that constitute targets of cell mediated immunity.

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Introduction

Primary biliary cirrhosis (PBC) and primary sclerosy cholangitis (PSC) are liver diseases with progressive destruction of bile ducts with a probable autoimmune aetiology. Knowledge of the underlying immune mechanisms in PBC is extensive; the putative antigen has been identified as an epitope of PDC E2 that has been shown to be expressed on bile duct epithelia in PBC [18, 20]. Several effector mechanisms have been proposed: IgG and complement components have been found to be deposited on bile duct epithelial cells in PBC as a token of humoral immune response [10]. In agreement with this hypothesis is the finding of a predominant B-cell infiltration around the bile ducts in PBC [14]. However, a T-cell dependent immune reaction has been incriminated as the major mechanism of bile duct destruction and PDC E2-specific T cells have been isolated from livers with PBC [12].

Data on the immune reaction in PBS are sparse. The significance of p-antinembophil cytoplasmic antigen (ANCA) in the serum of patients with PSC has not been evaluated. Some authors have found a high activity of NK cells in the livers of PSC patients [8], however, their relevance is still obscure.

The morphology of the two diseases shows both common and disparate features: whereas both display enlargement of portal tracts with a predominantly mononuclear inflammatory infiltrate, the typical lesions of bile ducts in PBS consist of disruption of the basement membrane of the bile ducts, granuloma formation and overt destruction of biliary epithelium. In PSC, in contrast the biliary basement membrane is intact and thickened and bile ducts are encircled and clasped by periductular collagen fibres. In morphological terms in PBC bile ducts are blown up, whereas in PSC the bile ducts are strangled by scarring collagen fibres.

In order to see if there are distinguishing immunological features that could explain liver morphology, biopsies from both groups were subjected to immunostaining for epitopes that might substantiate a role for bile duct epithelial cells as targets for cell mediated immune attacks.

Materials and methods

Twenty-three patients with confirmed PBC were included with available biopsies (21 females, 2 males). The diagnosis was confirmed by determination of antimitochondrial antibody type 2 in the serum and by histopathology (histological staging showed 2 with stage 1, 6 with stage 2, 12 with stage 3 and 4 with stage 4; according to Scheuer [16]). The PSC patients were a group of 12 with established diagnosis (6 females, 6 males). They all had typical lesions by imaging endoscopic procedures with strictures and narrow segments in the large bile ducts ducts and typical histological lesions. Nine of them showed p-ANCA in the serum. Fresh frozen liver biopsies were available from all patients. All biopsies had at least four partal tracts with discernible and adequate bile ducts. Cryostat sections were performed and incubated with the following antisera against HLA-I, HLA-II, CD2, CD4, CD8, CD28, CD58 (LFA) [lymphocyte function-associated antigen 3], CD80 (B1 BB7), CD95 (Fas), transforming growth factor, (TGF)-β, interferon (IFN)-γ, interleukin (IL)-2, tumour necrosis factor (TNF)α, IL-4, IL-10. Antibodies and their working dilutions are summarized in Table 1. For negative controls the primary antibodies were omitted and additionally an irrelevant monoclonal antibody directed against the core antigen of hepatitis B virus was used.

The sections were read without knowledge of their identity and scored semiquantitatively on a scale 0–3 taking staining intensity and number of positive cells into account. The assessment included bile ductules, interlobular bile ducts (20–100 μm diameter) and medium-sized ducts (>100 μm in diameter). The other structures assessed included endothelium, hepatocytes, sinusoidal lining cells and infiltrating inflammatory cells. Mononuclear cells of the inflammatory infiltrate positive for CD2, CD28, IFN-γ, IL-2, IL-4, IL-10 and TNF-α were counted and calculated as a percentage of the total amount of cells of each of the portal tracts in the sections.

Table 1 Antibodies used (*IL* interleukin, *TGF* transforming growth factor, *IFN* interferon, *TNF* tumour necrosis factor)

Type of antibody	Dilution	Source
CD2	1:100	Virotec
CD4	1:200	DAKO, Hamburg
CD8	1:200	Immunotec
CD28	1:10	Dianova
CD58	1:10	Autschbach et al. 1991 [2]
CD80	1:10	Dianova
CD95	1:50	Immunotec
HLA-I	1:100	Dianova
HLA-DR	1:200	Becton and Dickinson
IL2	1:50	Dianova
TGF-β	1:100	Genzyme, Cambridge, Mass.
IFN-γ	1:500	Dienes et al. 1991 [6]
IL4	1:10	TEBU, Frankfurt/M.
IL10	1:10	TEBU, Frankfurt/M.
TNF-α	1:50	Boehringer Mannheim

Results

In PBC almost all of the bile ducts were positive for HLA-I with a membranous staining of the bile duct epithelium. Anti-HLA-II also showed binding to the biliary epithelial cells in 21 of the 23 biopsies with the interlobular ducts being the most positive.

In the PSC specimens the reaction of the biliary epithelium was less pronounced. The expression of HLA-I antigens was weaker and less frequent. Nine of the 12 biopsies were positive. HLA-II was displayed on the majority of the specimens (8 of 12), but the staining was mostly confined to the apical segment of the membrane of the epithelial cells.

CD58 (LFA3) was expressed on the epithelial cells in the interlobular bile ducts (Fig. 1) in 18 of 23 cases in PBC. Some lymphocytes and endothelial cells were also reactive, and in 2 cases the periportal hepatocytes also showed membranous staining for LFA3. In PSC, however, only very few bile ducts displayed the antigen. Only 4 of 12 biopsies had a positive reaction in the interlobular bile duct epithelium and a couple of lymphocytes were positive as well.

The CD80 (B7 BB1) epitope was detected on bile duct epithelial cells (Fig. 2), some Kupffer cells and on some hepatocytes in active cases of PBC (stage 2 and 3). In more detail; 16 of 23 biopsies in PBC showed a weak to moderate distinct staining of bile duct epithelial cells. The plasma membrane gave a positive reaction on apical and lateral segments. In PSC only 2 of 12 biopsies contained bile ducts with the same pattern (Fig. 3), whereas the others were negative.

The pattern for CD95(Fas) was similar to that of CD80. In PBC 17 of 23 biopsies showed bile ducts positive (Fig. 4) for this epitope. In the same biopsy not all ducts stained evenly positive. The interlobular specimens in particular were decorated by the antibodies and mononuclear cells and hepatocytes in the periportal areas reacted in the same manner.

In PSC only 5 of the 12 biopsies showed positive bile ducts for the CD95 (Fas) epitope (Fig. 5). In three of the biopsies with negative staining for bile ducts; however, hepatocytes reacted weakly to the antibody. Lymphocytes were positive in almost all cases (the staining results for bile duct epithelia is summarized in Table 2).

Fig. 1 Florid bile duct lesion in primary biliary cirrhosis (PBC). ▶ Bile duct epithelial cells are positive for CD58 (lymphocyte function-associated antigen 3). Periportal hepatocytes display also a membranous staining. (×420)

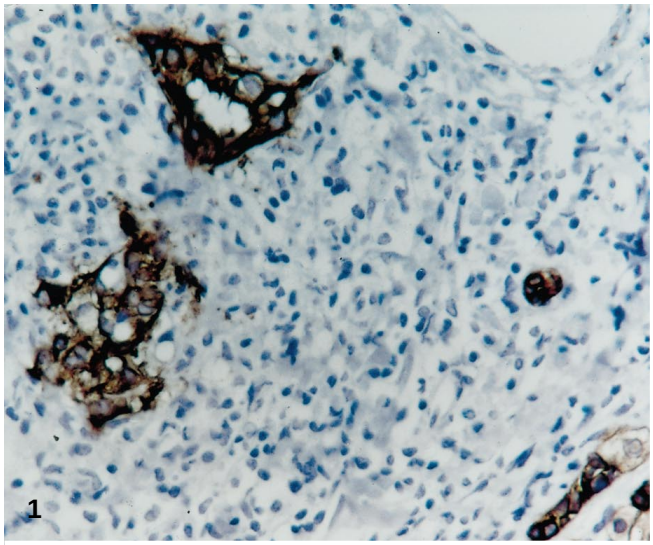
Fig. 2 CD80 in bile duct epithelia in PBC. There is only a faint reaction in a intermediate bile duct (×420)

Fig. 3 Positive staining for CD80 on a bile duct undergoing destruction in PBC. (×380)

Fig. 4 CD95 is positive on epithelia of a small bile duct in PBC. (×420)

Fig. 5 CD95 on several small bile ducts in primary sclerosing cholangitis gives only a mild reaction. (×420)

Fig. 6 There are numerous lymphocytes stained for CD28 in the portal tract of a biopsy from a patient with PBC. Some of them are infiltrating the bile duct epithelia (×420)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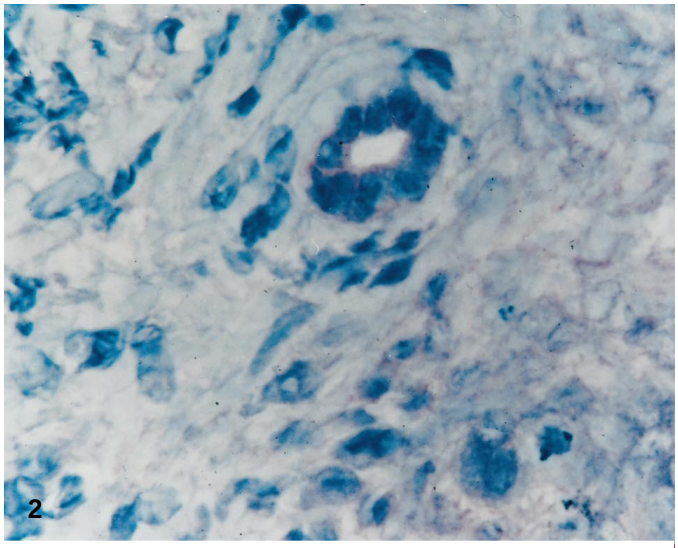
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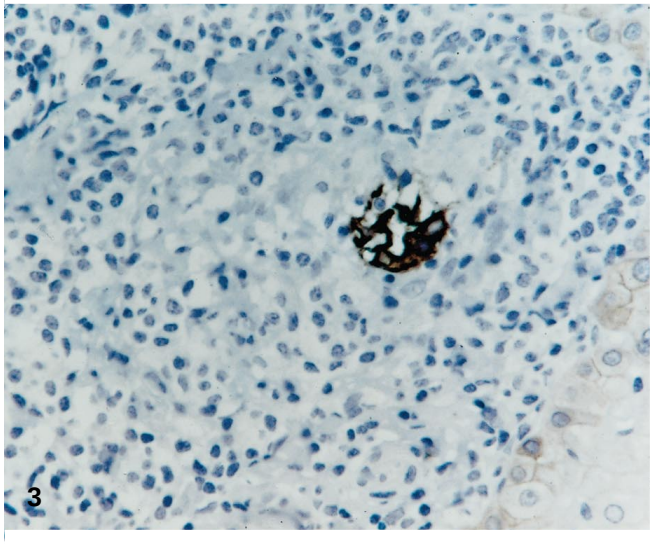
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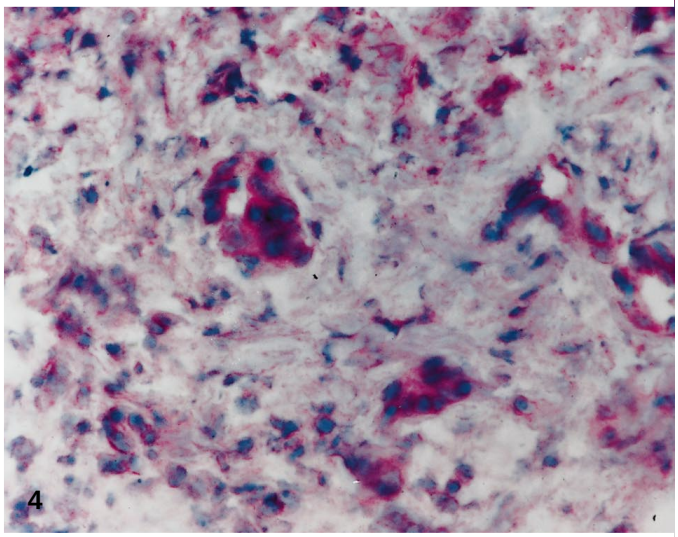
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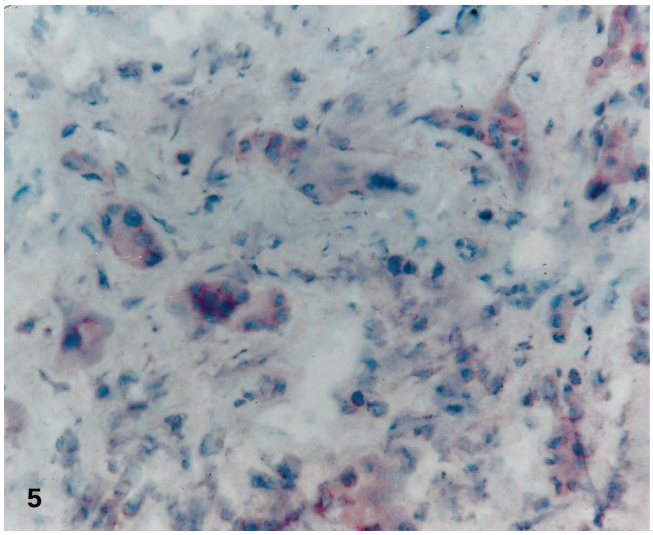
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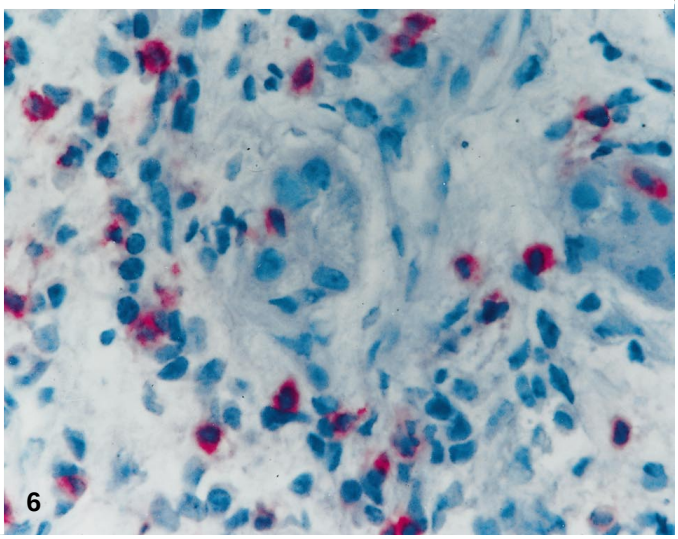
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Table 2 Staining scores of bile duct epithelia[a] (*PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis)

[a] Results are given as mean score (range)
[b] Subgrouping of biopsies into the four stages is disregarded in this table for reasons for clarity; instead mean values are given

PBC (*n*=23)			Antigen	PSC (*n*=12)		
Ductules	Interlobular	Medium		Ductules	Interlobular	Medium
2.6 (2–3)	2.9 (2–3)	2.5 (2–3)	HLA-I	1.4 (0–3)	1.5 (0–2)	1.1 (0–2)
2.5 (2–3)	2.9 (2–3)	2.6 (1–3)	HLA-II	1.1 (0–2)	1.0 (0–1)	0.9 (0–1)
1.8 (1–2)	2.2 (0–3)	2.3 (1–3)	CD58 (LFA3)	0.4 (0–1)	0.3 (0–1)	0.3 (0–1)
1.2 (0–2)	1.9 (2–3)	1.7 (1–3)	CD80 (BB7)	0.2 (0–1)	0.3 (0–1)	0.2 (0–1)
1.9 (1–3)	2.5 (1–3)	2.1 (1–3)	CD95 (Fas)	0.4 (0–1)	0.5 (0–2)	0.3 (0–1)

Subtyping of lymphocytes by antibodies against CD4 and CD8 epitopes yielded a majority of CD4 positive T-cells in both PBC and PSC. In PBC 49% of T-cells stained positive for CD4 and 26% for CD8 when the late stages 3 and 4 were estimated. In stages 1 and 2 the correlation was slightly lower. In PSC the total amount of lymphocytes was lower compared to PBC. However, the relation of CD4 to CD8 was similar: 51% of 28%. Most of the lymphocytes were concentrated to the portal tracts. CD4-positive lymphocytes were also observed infiltrating the bile duct epithelial cells in PBC.

CD2, which is the natural ligand to CD58 (LFA3), was positively stained in the vast majority of the inflammatory infiltrates in both PBC and PSC. Antibodies to CD28 stained a substantial part of the lymphocytic infiltrates in PBC and PSC. In PBC the CD28-positive (Fig. 6) cells made up about 45% and in PSC 41% of the mononuclear cells. In PBC and to a lesser degree in PSC, Kupffer cells also reacted with this antibody, displaying a diffuse pattern.

The patterns of cytokines of the lymphocytes showed a high percentage of IFN-—positive cells in PBC (38% of lymphocytes) and in PSC (32% of lymphocytes). IL-2-positive cells represented 29% in PBC and about 30% in PSC. TNF-α was less frequently detected making up only 23% in PBC and 19% in PSC. The cytokines of the Th2 lymphocyte subtype (IL-4 and IL-10) were only secondary with IL-4-staining in 16% of the lymphocytes in PBC and 18% in PSC. IL-10-positive lymphocytes were even less numerous with 15% only in PBC and 10% in PSC.

Kupffer cells also stained for cytokines in most cases of both diseases with TNF-α being the most abundant among the cytokines tested. TGF-β was another cytokine that was analysed in the tissues. In biopsies with PBC and PSC Kupffer cells were positive in all cases, however, to a variable degree. In 10 of the 11 biopsies of PSC the bile duct epithelial also displayed conspicuous staining for TGF-β whereas in PBC only 7 out of the 23 cases had a similar reaction.

Discussion

PBC and PSC are regarded as chronic inflammatory liver diseases of autoimmune aetiology [7]. In PBC knowledge of the immune mechanisms is quite advanced regarding the target antigens on bile duct epithelial cells and the steps of the immune response involved [4, 8, 10, 14, 18, 20], but for PSC few data are available. The diseases have different morphology although they have a common final pathway with loss of bile ducts. In PBC there is overt destruction of the bile duct epithelium with disruption of the basement membrane, sometimes associated with granuloma formation, infiltration of the bile duct epithelial cells by lymphocytes and necrosis of bile duct epithelium. In PSC the inflammation is generally at a low level, there is no overt bile duct epithelial necrosis and the basement membrane is often thickened instead of destroyed.

In order to see if the different morphology was reflected in differences in immune factors in the tissue (espially if bile duct epithelium can present as target cells of immune attacks as can be suggested by the histopathology in PBC) we performed an analysis of immune-relevant factors in the liver tissues. CD8 lymphocytes have been thought to the possible effector cells [20]. However, other groups using different techniques [13] or analysing larger groups of patients [10] demonstrated a predominance of CD4-positive lymphocytes which is in keeping with the results of the present study. Subtyping of CD4-positive lymphocytes into Th1 and Th2 populations by cytokine analysis yielded conflicting results. Whereas Spengler et al. [17] found a defect in IL-2-producing lymphocytes, Löhr et al. [13] identified a majority of Th1-lymphocytes after isolation from the tissue and determination of the cytokines by cell culture techniques. Their results showed that IFN-γ was the most abundant factor followed by IL-2 and TNF-α. These results could be confirmed in the present study by typing the lymphocytes in situ with antibodies against the different cytokines. IL-4 and IL-10 indicated that Th2 lymphocytes were less frequent than IFN-γ, IL-2 and TNF-α. This relationship was found in PBC and PSC although the absolute numbers were lower in PSC.

Summarizing the data from the literature appears to indicate that the potential effector cells in PBC and PSC are CD4-positive lymphocytes belonging mainly to the Th1-subtype and the activated memory T-cell population positive for CD45RO [11, 14].

The importance of B cells in the pathogenesis of PBC has already been evaluated [14,19], however, a relevant role of B cells would require a predominant Th2 lymphocyte function, which we cannot confirm. The targets of the immune attack in PBC have been identified as the PDC E2 molecules that show enhanced expression on

bile duct epithelium in PBC but not on normal bile ducts and also not in PSC [20]. There is ample evidence that bile duct epithelial cells in PBC may act as target cells. They display HLA class I and II epitopes on the plasma membrane [5, 20]. The also may express adhesion molecules ICAM-1 in the late stage [5] in the interlobular branches [1] and VCAM [20]. The bile duct epithelia in PSC seem to behave in the same manner with expression of HLA-DR [5] and ICAM-1. Data on VCAM have not been reported.

The effector-target cell binding complex encompasses further epitopes and ligands to provide a tight connection between the lymphocytes and targets and additional activation of the effector cells. These are mainly CD58 (LFA3) and CD80 (B1 BB7). The binding of CD58 to CD2 mediates adhesion and signal transduction for T-cell activation [3], thus constituting an essential component of immune response in vivo. We have demonstrated for the first time that this molecule is positive on bile duct epithelium in PBC, however, it is expressed only in a minority in of cells in PSC CD2-positive lymphocytes are in the majority. The display of these epitopes is correlated with the degree of inflammation and stronger visible in stages 2 and 3 of PBC than in the end stage. In PSC only a few cases (4 of 12) showed positive bile ducts that stained for LFA3.

CD80 (B1 BB7) is another molecule that mediates adhesion to CD28-positive lymphocytes and provides important costimulatory signals for T-cell proliferation [9]. Previous studies on this important epitope on isolated biliary epithelium showed negative results [11] even after stimulation with IFN-γ. Immunohistology on a small group of liver biopsies also gave negative staining. However, in the present experiment, in 15 biopsies of 23, the bile duct epithelia were positive for the anti-CD80 antibodies. The positive reaction was only weak-to-moderate and tended to be visible only in stages 2 and 3 of the disease on interlobular bile ducts with a few exceptions on the medium-sized ones. In PSC only 2 of 12 biopsies showed an equal positive reaction. This diverging result from the other study may be due to a different antibody but also to the small number (5 patients) examined.

In order to explore whether the bile duct epithelial cells may undergo apoptosis via the Fas-mediated pathway, we looked for the expression of CD95, a prerequisite for this immune-mediated mode of cell death. The medium-sized and interlobular bile ducts in particular displayed this epitope on their epithelial cells in PBS (19 of 23), whereas small ductules and larger medium-sized ducts gave weaker or negative results. In PSC only a minority of the specimens were positive (5 of 12) with only a mild positive reation on the bile ducts and on lymphocytes. These data in agreement with a previous report [15] and further support the notion that in PBC bile duct epithelia may be destroyed due to a cell-mediated immune attack.

The present findings give support to the concept that bile duct epithelial cells in PBC may present as target cells for cell-mediated immune mechanisms with CD4-positive lymphocytes of the Th1 subgroup being the predominant effector cells.

In PSC, however, most of the cells lack, or express only weakly, the essential epitopes for a function as targets-CD58 (LFA3), CD80 (B1 BB7) and CD95 (Fas). These immune patterns are in accordance with the different morphology of both diseases. There are still open questions as to the aetiology of granulomas in PBC and the mechanisms of the disappearance of bile ducts in PSC. Excess of extracellular matrix formation periductular fibrosis) may be one of the main factors.

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References

1. Adams DH, Hubscher SG, Shaw J, Johnson GD, Babbs C, Rothlein R, Neuberger JM (1991) Increased expression of intercellular adhesion molecule 1 on bile ducts in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 14:426–431
2. Autschbach F, Meuer SC, Moebius U, Manns M, Hess G, Meyer zum Büschenfelde K-H, Thoenes W, Dienes H-P (1991) Hepatocellular expression of lymphocyte function-associated antigen 3 in chronic hepatitis. Hepatology 14:223–230
3. Berke G (1994) The functions and mechanisms of action of cytolytic lymphocytes. In: Paul WE (ed) Fundamental immunology. Raven Press, New York
4. Björkland A, Festin R, Mendel-Hartvig I, Nyberg A, Lööf L, Totterman TH (1991) Blood and liver-infiltrating lymphocytes in primary biliary cirrhosis: increase in activated T and natural killer cells and recruitment of primed memory T cells. Hepatology 113:1106–1111
5. Bloom S, Fleming K, Chapman R (1995) Adhesion molecule expression in primary sclerosing cholangitis and primary biliary cirrhosis. Gut 36:604–609
6. Dienes HP, Hess G, Wörsdörfer M, Rossol S, Gallati H, Ramadori G, Meyer zum Büschenfelde KH (1991) Ultrastructural localization of interferon producing cells in the liver of patients with chronic hepatitis B. Hepatology 13:321–326
7. Freilich BL, Hu K-Q, Vierling JM (1994) Immunopathogenesis of autoimmune hepatobiliary diseases. Liver 10:257–268
8. Hata K, Van Thiel DH, Herberman RB, Whiteside TL (1992) Phenotypic and functional characteristics of lymphocytes isolated from liver biopsy specimens from patients with active liver disease. Hepatology 15:816–823
9. Janeway CA Jr, Bottomly K (1994) Signals and signs for lymphocyte responses. Cell 76:275–285
10. Krams SM, Van de Water J, Coppel RL, Esquivel C, Roberts J, Ansari A, Gershwin ME (1990) Analysis of hepatic T lymphocyte and immunoglobulin deposits in patients with primary biliary cirrhosis. Hepatology 12:306–313
11. Leon MP, Kirby JA, Gibbs P, Burt AD, Bassendine MF (1995) Immunogenicity of biliary epithelial cells: study of the expression of B7 molecules. J Hepatol 22:591–595
12. Löhr H, Fleischer B, Gerken G, Yeamann SJ, Meyer zum Büschenfelde KH (1993) Autoreactive liver infiltrating T cells in primary biliary cirrhosis recognize inner mitochondrial epitopoes and the pyruvate dehydrogenase complex. J Hepatol 18:322–327
13. Löhr HF, Schlaak JF, Gerken F, Fleischer B, Dienes H-P, Meyer zum Büschenfelde K-H (1994) Phenotypical analysis and cytokine release of liver-infiltrating and peripheral blood T lymphocytes from patients with chronic hepatitis of different etiology. Liver 14:161–166

14. Nakanuma Y (1993) Distribution of B lymphocytes in nonsuppurative cholangitis in primary biliary cirrhosis. Hepatology 18:570–575
15. Okazaki M, Hino K, Fujii K, Hanada H, Nakanishi Y, Kanazawa S, Kobayashi N, Okita K (1995) Immunohistochemical study of Fas antigen in liver of patients with chronic hepatitis and autoimmune liver disease. Int Hepatol Comm 3:285
16. Scheuer PJ, Lefkowitch JH (1994) Liver biopsy interpretation. WB Saunders, London
17. Spengler U, Moller A, Jung M, Messer G, Weiss EH (1992) T lymphocytes from patients with primary biliary cirrhosis produce reduced amounts of lymphotoxin, tumor necrosis factor and interferon-γ upon mitogen stimulation. J Hepatol 15:129–135
18. Van de Water J, Turchany J, Leung PSC, Lake J, Munoz S, Surh CD, Coppel R, Ansari A, Nakanuma Y, Gershwin ME (1993) Molecular mimicry in primary biliary cirrhosis. J Clin Invest 91:2653–2664
19. Yamada G, Hyodo I, Tobe K, Mizuno M, Nishihara T, Kobayashi T, Nagashima H (1986) Ultrastructural immunocytochemical analysis of lymphocytes infiltrating bile duct epithelia in primary biliary cirrhosis. Hepatology 6:385–391
20. Yasoshima M, Nakanuma Y, Tsuneyama K, Van de Water J, Gershwin ME (1995) Immunohistochemical analysis of adhesion molecules in the micro-environment of portal tracts in relation to aberrant expression of PDC-E2 and HLA-DR on the bile ducts in primary biliary cirrhosis. J Pathol 175:319–325