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Primary biliary cirrhosis (PBC): antigen-presenting cells differ in their distribution in early and late stage PBC and involve the ductal, but not the ductular compartment

D. Rontogianni[1], H. Gerber[2] and A. Zimmermann[2]

[1]Department of Pathology, Evangelismos Hospital, Ipsilantou, Athens, Greece and

[2]Institute of Pathology of the University, Bern, Switzerland

Summary. We have studied the distribution patterns of antigen-presenting cells (APCs) in different stages of primary biliary cirrhosis (PBC). 58% of cases with early PBC (stages I and II) exhibited dendritic APCs (S-100$^+$, HLA-DR$^+$, KiMlp$^+$) in bile duct epithelia. In contrast, APCs, were not detected in ductular proliferations occurring in late PBC (stages III and IV), but occurred in portal tracts and piecemeal necroses. There was a correlation between the presence of APCs and HLA-DR expression in bile ducts but, in contrast to former studies, we noted a heterogeneous ductal HLA expression. These observations support the hypothesis that: 1) APC distribution in PBC may change as a function of stage, involving hepatic parenchyma in late PBC; 2) ductular epithelia may not represent a target for immune attack, because APCs do not accumulate in these structures; and 3) HLA expression in bile ducts may be heterogeneous, suggesting one mechanism why bile duct destruction in PBC does not take place in a synchronous way.

Key words: Antigen-presenting cells, Primary biliary cirrhosis

Introduction

Many features of primary biliary cirrhosis (PBC) indicate that it represents an autoimmune disease, and efforts have been made to identify cellular antigens acting as possible inducers of immune reactions (Sherlock, 1989). Antimitochondrial antibodies (AMA) were found to be directed against subunits of mitochondrial multienzyme complexes (Van de Water et al., 1988; Yeaman et al., 1988; Fregeau et al., 1990; Surh et al., 1990; Yoshida et al., 1990), leading to accumulating information on possible antigen(s) involved. In contrast, cellular effector mechanisms of cell damage in PBC and their change as a function of stage are not yet fully understood. In particular, the timely evolution of the tissual distribution of antigen-presenting cells in different stages of PBC has not been systematically analyzed.

Antigen-presenting cells (APCs) play a central role in the afferent limb of the immune response, representing the outermost sentinels of immune reactions driven by distinct antigens. It may, therefore, be anticipated that APCs can be detected in the tissues at critical immunological contact sites. In fact, APCs with a dendritic morphology (dendritic cells, DCs; McKenzie et al., 1989; King and Katz, 1990) and expressing S-100 protein have been observed inside the bile duct profile in PBC (Demetris et al., 1989), as have macrophages as another member of accessory cells enabled to handle and present antigens (Tobe, 1982; Tobe et al., 1982). However, accumulation of APCs at sites of immunological tissue damage may represent a transient feature and may vary as a function of time for the following reasons. Firstly, the early critical interaction between (auto)antigen, APCs and effector cells may occur at a time point that is remote from the clinical response. Secondly, primed lymphocytes with memory are longlived and circulate in blood and lymph, and can thus be recruited from sets residing at a distance from the target tissue. Thirdly, antigen recognition depends on the local expression of HLA gene products, which may fluctuate with time. Therefore, APCs accomplishing antigen presentation to effector lymphocytes may require their presence only at the time of acquisition, i.e. in earlier phases of disease. In order to test this hypothesis the present study was performed to analyze the tissual distribution of DCs/APCs as a function of the stage of PBC, and to extend investigations from bile ducts to their ductular tributaries. Furthermore, we studied the relationship between the time and spatial distributions of APCs, HLA expression in bile duct cells, and intermediate filament expression in bile duct and

Offprint requests to: Prof. Arthur Zimmermann, Institute of Pathology of the University, Murtenstrasse 31, CH-3010 Bern, Switzerland

Table 1. Summary of relevant patient data.

PATIENT	SEX/AGE (yrs)	HEPATOMEGALY	SPLENOMEGALY	AUTO Abs			IgM	LFT	
				AMA	ANA	SMA	g(l)	GEC[1]	GEC[2]
1	F/67	+	+	+	-	-	3.8	254	4.9
2	F/66	+	-	+	-	-	4.8	240	5.3
3*	F/34	+	+	+++	+	-	10.5	385	7.7
4	F/58	+	-	++	+	-	16.3	416	5.5
5	F/53	+	-	+	-	-	10.1	462	6.3
6	F/65	+	-	+	-	-	20.4	424	7.1
7	F/46	+	-	+	-	-	nk	421	6.9
8	F/68	+	nk	+	-	-	5.5	244	5.3
9	F/71	+	nk	++	nk	nk	10.9	237	4.2
10	F/47	+	nk	+	+	nk	3.3	362	6.3
11*	F/62	+	+	+	nk	nk	9.4	241	4.7
12*	F/55	+	+	+	-	-	10.7	359	8.1
13	F/33	+	+	+	nk	nk	2.3	372	7.3
14	F/55	+	+	+	-	-	5.3	199	4.3

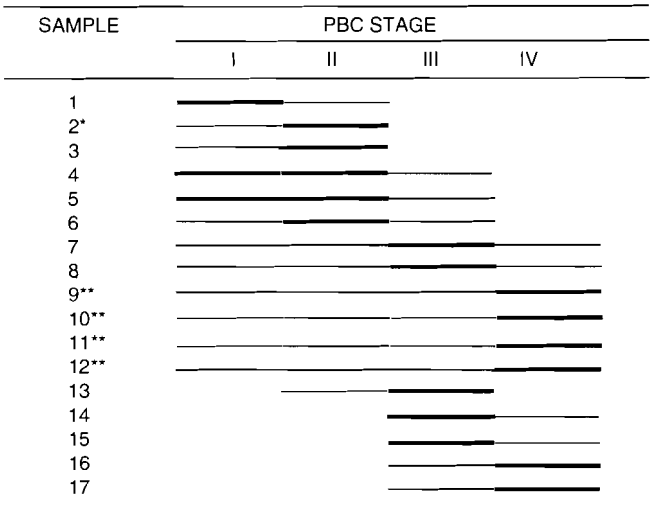
*: more than one biopsy per patient analyzed; AUTO Abs: autoimmune antibodies; AMA: antimitochondrial antibodies; ANA: antinuclear antibodies; GEC: galactose elimination capacity; GEC[1]: mg/min (normal range, 370-640); GEC[2]: mg/min/kg (normal range, 6.8-9.1); LFT: liver function tests; nk: not known; SMA: anti-smooth muscle antibodies.

ductular cells.

Materials and methods

Liver tissue samples were obtained from 14 female patients with PBC diagnosed according to generally accepted clinical and biochemical criteria (James et al., 1983). The relevant patient data are summarized in Table 1. Histological staging is listed in Table 2. As a working formulation, PBC stages I and II were defined as early stage PBC, and stages III and IV as late stage PBC. Tissue examined included 13 biopsies (11 percutaneous needle biopsies and 2 operative wedge biopsies) and 4 livers obtained at the time of transplantation. 11 liver samples showing changes different from that of PBC served as random controls (3 cases of chronic alcoholic liver disease, 3 cases of liver cirrhosis of unknown cause (one with ductular proliferations), one case of chronic active hepatitis C, one case with fatty change and cholestasis (probably due to toxic damage), 2 cases with minor hepatic changes, and one pediatric case (not further specified disorder of psychomotor development)). Furthermore, 20 liver tissue samples of 14 patients with stenosing bile duct disease different from PBC (6 benign structures, 7 neoplastic stenoses, 1 *Echinococcus alveolaris*) were included in this study. For control of HLA immunoreactivity, human liver allograft biopsies were used. For conventional light microscopy, tissue was fixed in 4% neutral buffered formaldehyde, embedded in paraffin, and processed to 5 µm sections. Staining included haematoxylin-eosin, PAS, reticulin, Van Gieson's and Prussian blue. Immunostaining was performed at room temperature with primary antibodies listed in Table 3, and using the avidin-biotin peroxidase complex (ABC) technique (Hsu et al., 1981). In brief, paraffin sections mounted on glass slides coated with polyvinyl acetate glue (Järvinen and Rinne, 1983) were

Table 2. Histological staging.



*: open wedge biopsy; **: hepatectomies; ——— : histological changes corresponding to various stage; ▬▬ : predominant stage.

deparaffinized, hydrated, digested with proteases (see Table 3 for details) and immersed in 5% skimmed milk (Johnson et al., 1984) to block non-specific binding of reagents. The sections were incubated overnight with primary antibodies, and further incubated one hour each with biotinylated rabbit anti-mouse or swine anti-rabbit immunoglobulin antisera (Dakopatts, 1:100) and ABC reagents (Dakopatts, 1:200). Peroxidase activity was visualized with diaminobenzidine as chromogen (Ruchti et al., 1984), the nuclei stained with haematoxylin and the sections mounted in aqueous polyvinyl alcohol medium (Freer, 1984). Incubations without specific antibody served as negative controls.

Results

Spatial distribution of APCs with dendritic morphology

Cells with dendritic morphology (DCs) were S-100$^+$ and HLA-DR$^+$ and reacted with the monoclonal antibody KiMlp (Table 3). DCs with this phenotype were found within the epithelial lining of septal and interlobular bile ducts in 7/12 (58%) cases with early stage PBC (Table 4). Bile ducts harbouring DCs were either surrounded by inflammatory cells, showed little or no evidence of inflammation, or were already damaged. Cases 13 to 17, corresponding to late stage PBC, did not show bile ducts in the biopsies.

S-100 protein showed a strong diffuse cytoplasmic and nuclear staining (Fig. 1), whereas the KiMlp antibody resulted in a strong granular cytoplasmic reaction. The cytoplasm of DCs was stained with HLA-DR antibody similarly to KiMlp (Fig. 2).

DCs were also observed in small interlobular bile

Table 3. Primary antibodies used in this study.

DESCRIPTION OF ANTIBODY	DILUTION	SOURCE
Monoclonal (mouse):		
L26 (CD20), B cells	1:100[b]	Dakopatts
UCHL1 (CD45R0), T cells	1:50[b]	Dakopatts
Leu-7 (CD57), NK cells	undiluted[a]	Becton Dickinson
KiB1p, follicular dendritic cells	1:800[a]	Institute of Pathology, Kiel, FRG
KiM1p, monocytes/macrophages	1:5000[a]	Institute of Pathology, Kiel, FRG
HLA-DR, MHC class II antigen	1:50[b]	Dakopatts
CAM 5.2, cytokeratins 8, 18, 19	undiluted[a]	Becton Dickinson
K_S 19.1 cytokeratin 19	1:100[a]	Progen
Vimentin	1:10[b]	Dakopatts
Polyclonal (rabbit):		
Protein S-100	1:200[a]	Dakopatts
ß2-Microglobulin, MHC class I antigen	1:1000[a]	Dakopatts

[a]: protease digestion in 0.02% trypsin (Difco, Trypsin 1:250) at 37 °C for 10 minutes. [b]: no protease digestion.

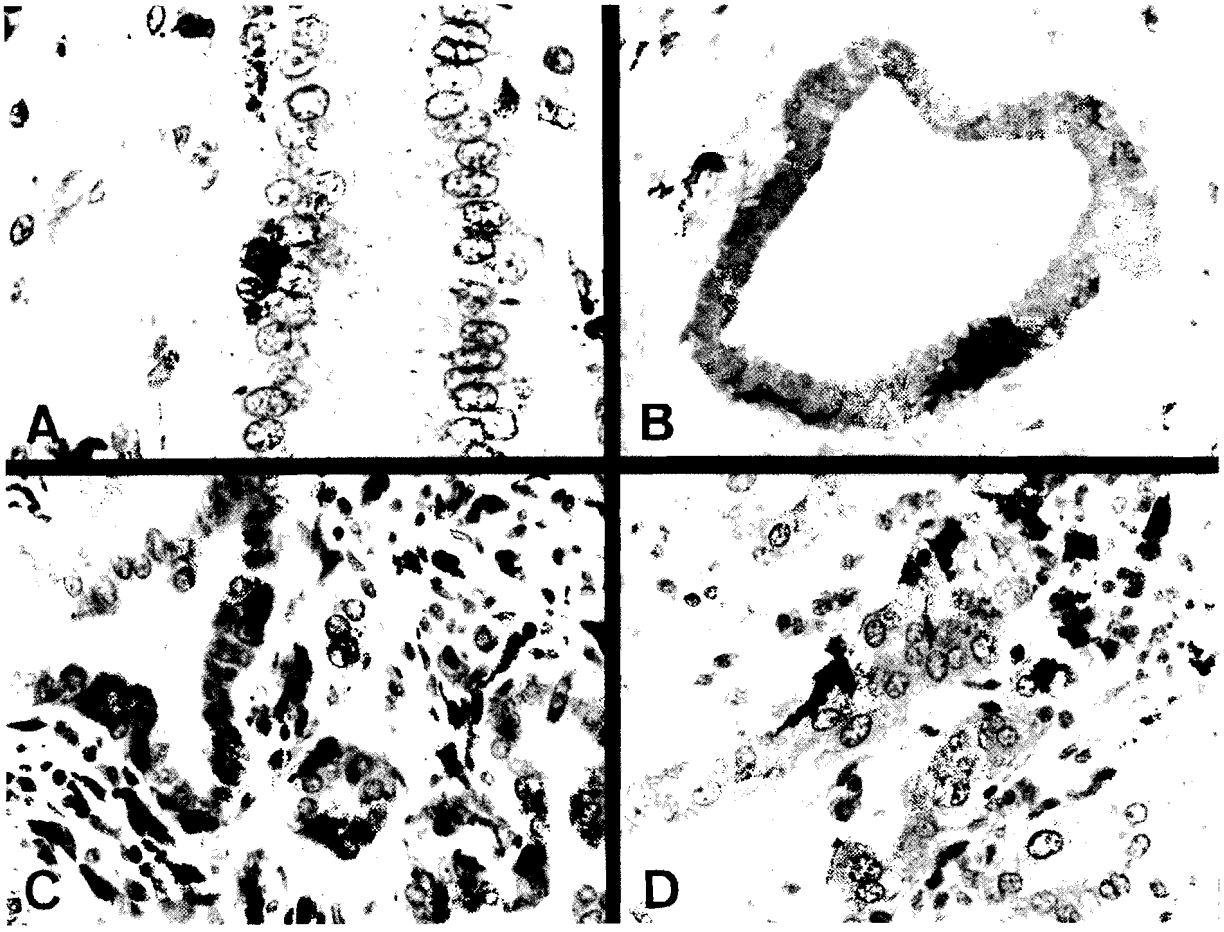


Fig. 1. Intraepithelial localization of S-100 and KiMlp cells with dendritic morphology in septal bile ducts. **A and C:** KiMlp$^+$ cells; **B and D:** S-100$^+$ cells. KiMlp$^+$ cells show a distinct and granular reaction product and are usually located in basal parts of the epithelial lining. Note that cells with a similar morphology and immunophenotype are seen within the periductal infiltrate. S-100$^+$ cells exhibit a diffuse cytoplasmic staining and may also express nuclear staining (D). The dendritic morphology is usually better visualized in S-100 preparation. Immunostain. x 553

ducts, even though their frequency was less in this duct category than in larger intermediate and in septal ducts. In contrast, DCs (S-100$^+$, HLA-DR$^+$, KiMlp$^+$) were not found within the epithelial lining of ductular proliferations which start to occur in stage II (Scheuer, 1967; Sherlock, 1989), but were encountered in larger amounts in late stage PBC.

Only few DCs with the same immunophenotype were observed in the interstitium of portal tracts in early stage PBC, intermingled with an infiltrate mainly consisting of UCHL1-positive lymphocytes, and some KiMlp$^+$ and HLA-DR$^+$ cells of other than dendritic morphology (probably macrophages) were noted in portal tracts. In late stage PBC, the majority of APCs and macrophage-like cells were localized in areas of piecemeal necrosis.

Independent of the stage, some KiMlp$^+$ and HLA-DR$^+$ cells with elongated, non-dendritic shape were found in a perivascular, subvenular, periductular and subcapsular distribution, (probably elongated forms of the macrophage system).

Very few dendritic cells (S-100$^+$) were found in the portal tracts of control biopsies, but in none of them S-100$^+$ cells were observed within bile duct epithelia or in the region of the limiting plate of either lobules or nodules. The quality of S-100 staining was assessed on the basis of clear staining of nerves represented in biopsies or explants.

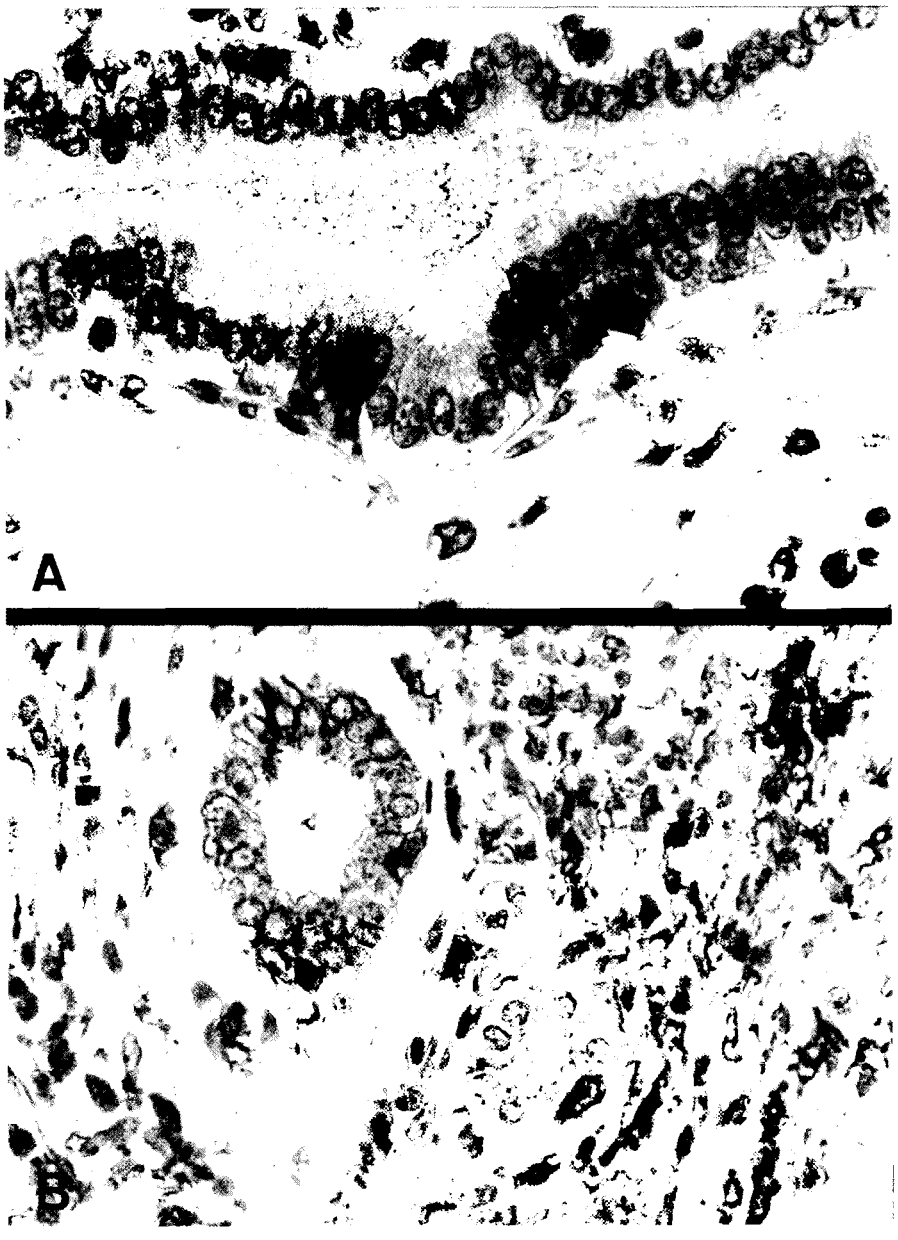


Fig. 2. Intraepithelial dendritic cells expressing HLA-DR. **A.** Shows the epithelial lining of a septal bile duct and an intraepithelial DC with a strong granular cytoplasmic reaction product. Cells with a similar immunophenotype but localized within the epithelial lining of intermediate and small interlobular bile ducts are represented in **B.** Immunostain. x 553

Expression patterns of HLA antigens

Beta-2-microglobulin (a component of HLA-A, B, C) was strongly expressed in the apical and lateral membranes, and weakly in the cytoplasm of cells of both uninvolved and already damaged bile ducts, and staining was variable in one and the same duct (Fig. 3). Interestingly, epithelia of ductular proliferations displayed beta-2-microglobulin thus staining in a similar way to interlobular bile ducts. Hepatocytes in lobular areas not involved by the disease were consistently negative for beta-2-microglobulin. Conversely, hepatocytes adjacent to inflamed portal tracts, and in particular in contact with piecemeal necroses, expressed the protein strongly in the cell membrane and weakly in the cytoplasm in 15/17 cases (88%). In one case with the most prominent cholestasis (case 12) expression of beta-2-microglobulin by hepatocytes appeared to be stronger in cholestatic pseudorosettes.

In contrast to beta-2-microglobulin, HLA-DR staining of biliary epithelia was found in involved ducts

Table 4. Frequency and distribution of dendritic cells in bile duct epithelia.

PATIENT	DENDRITIC CELLS WITHIN BILE DUCT EPITHELIA		
	S-100	KiMlp	HLA-DR
1	0^{P}	$+^{P}$	$+^{P}$
2	$++^{S}, +^{P}$	$++^{S}, +^{P}$	$+^{S}, +^{P}$
3	$+^{P}$	$+^{P}$	$+^{P}$
4	0^{P}	0^{P}	0^{P}
5	$++^{P}$	$+^{P}$	$+^{P}$
6	$+^{P}$	$+^{P}$	$+^{P}$
7	$+^{P}$	$++^{P}$	$++^{P}$
8	0^{P}	$+^{P}$	0^{P}
9	$0^{S,P}$	$+^{S}$	$+^{S}$
10	$+++^{S}, +^{P}$	$+++^{S}, +^{P}$	$++^{S}, +^{P}$
11	$+++^{S}, +^{P}$	$+++^{S}, +^{P}$	$+++^{S}, +^{P}$
12	$+^{S}$	$++^{S}, +^{P}$	$++^{S}, +^{P}$
13-17	-	-	-
Controls	0	0	0

^{S}: septal bile duct; ^{P}: portal bile duct; 0: no dendritic cell; +: very few dendritic cells; ++: few dendritic cells; +++: dendritic cells in many bile ducts; -: no septal or portal bile ducts.

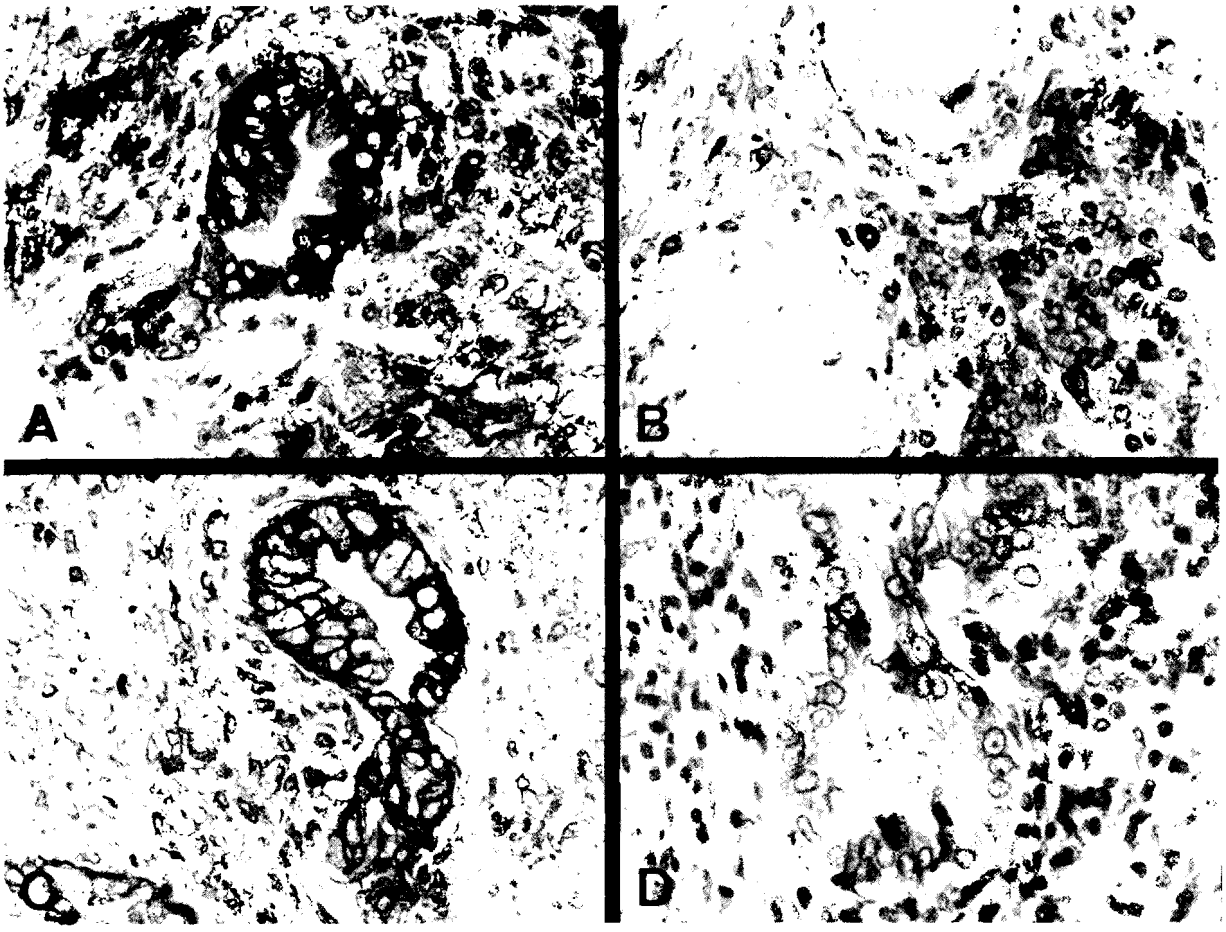


Fig. 3. Expression of beta-2-microglobulin in bile duct epithelia. **A.** The beta-2-microglobulin staining of this involved bile duct is variably expressed, in that one component (at the top) shows a much stronger staining than the duct segment shown at the bottom. **B.** HLA-DR expression in corresponding, involved bile ducts. **C.** This already damaged duct shows a strong beta-2-microglobulin expression mainly at the cell periphery. **D.** HLA-DR expression in corresponding bile ducts. ABC peroxidase stain. x 350

Table 5. Expression of HLA-DR, HLA-ABC, and vimentin in bile ducts (BD) and in newly-formed bile ducts (NFD) in relationship to the presence of dendritic intraepithelial cells.

PATIENT	DCs		HLA-DR		HLA-ABC		VIMENTIN	
	BD	NFD	BD	NFD	BD	NFD	BD	NFD
1	+	0	+	0	+	+	0	0
2	+	0	0-+++	0	+	+	0	++
3	+	0	0	0	+	+	0	0
4	0	0	0	0	+	+	0	0
5	++	0	0-+++	0	+-+++	+	0	++
6	+	0	0-+	0	+	+	0	++
7	+	0	0-+	0	+-+++	+	0	0
8	+	0	0-+++	0	+-+++	+	0	++
9	+	0	0-+++	0	+	+	0	++
10	+++	0	0-+++	0	+-+++	+	0	++
11	+++	0	0-+++	0	+-+++	+	0	++
12	++	0	0-+	0	+	+	0	++
13-17	NF	0	NF	0	NF	+	NF	++
Controls	0	NF	0	NF	0-+	NF	0	NF

0: no visible expression/no cells; +: weak expression/very few cells; ++: moderate expression/few cells; +++: strong expression/cells in many bile ducts; NF: not found.

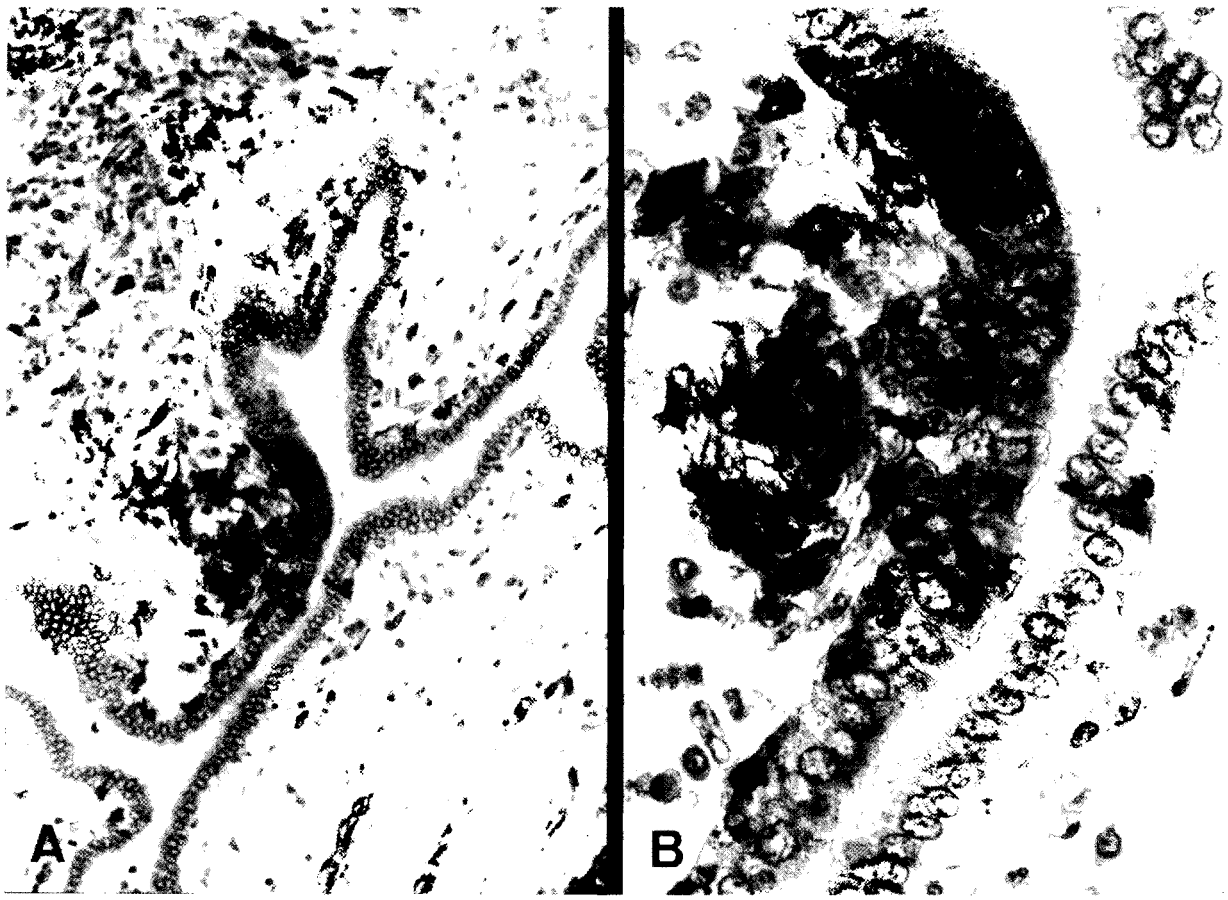


Fig. 4. HLA-DR expression in epithelial areas of bile ducts populated by dendritic cells (DCs). **A.** Focal epithelial HLA-DR staining in a septal duct. In the vicinity of this labelled area one observes dense round cell infiltrates. **B.** This epithelial area is also infiltrated by DCs. ABC peroxidase stain. A: x 175. B: x 553

only. Staining was found to vary between the cells of ducts in the same and in adjacent portal tracts (cases 1 to 12; Table 4), but HLA-DR expression was stronger in clearly damaged ducts, and was lacking in uninvolved ducts even if there was a massive lymphocytic infiltration of the respective portal tracts. In cases 1 and 2 and 5 to 12, but not in 3 and 4 (Table 4) bile ducts with intraepithelial HLA-DR$^+$ DCs usually also expressed HLA-DR in the epithelial lining, thus producing «spots» of colocalization of two features (Fig. 4). However, in cases 3, 5, 7 and 8 (two early and two late PBCs) DCs (HLA-DR$^{+)}$ were visualized in ducts not showing epithelial HLA-DR staining, even though other duct segments in the same portal tract expressed patchy staining and were surrounded by rather dense round cell infiltrates (Fig. 4a).

HLA-DR expression was never observed in epithelia of ductular proliferations or in hepatocytes.

No HLA-DR expression was observed in any of the epithelial cells in non-OLT controls, but was visualized in OLT biopsies. Bile duct epithelia of control biopsies strongly stained for beta-2-microglobulin.

Intermediate filament expression in ductular proliferations

As previously shown, epithelial cells of ductular proliferations gave a strong reaction with CAM 5.2 and CK 19 antibodies in all cases examined (Fig. 5). In contrast to septal and interlobular bile ducts ductular proliferations expressed both cytokeratins and vimentin, the latter being observed in 13/17 cases (76%; Fig. 5). In the control group with bile duct disease not related to PBC, vimentin-positive ductulus were found in 9/13 samples analyzed.

Discussion

The first aim of the present study was to address the question as to whether the tissual distribution of antigen-presenting cells (APCs; McKenzie et al., 1989; King and Katz, 1990) in the liver of patients with PBC changes as a function of the progression of disease. APCs with a dendritic morphology have previously been reported to occur inside the bile duct profiles in PBC (Demetris et

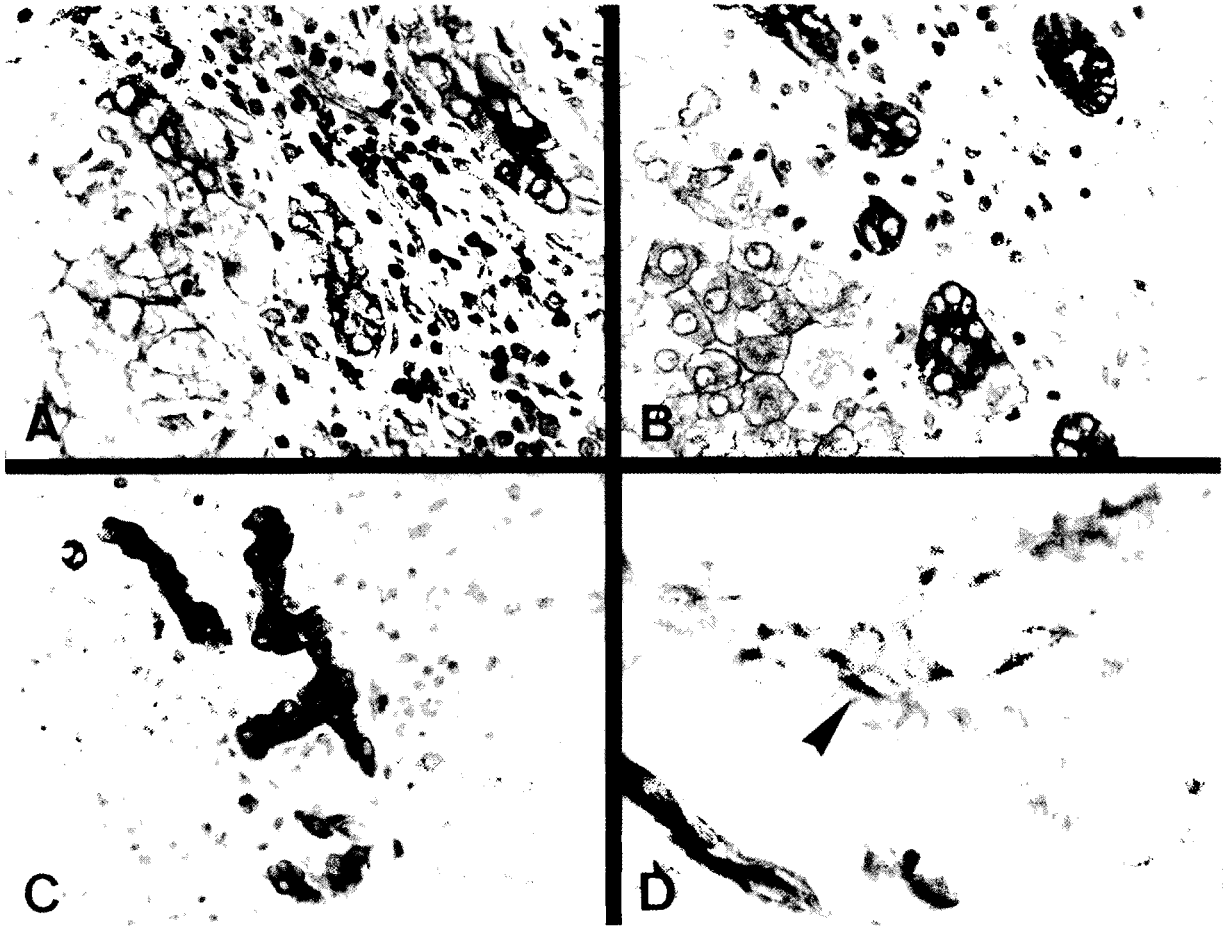


Fig. 5. Immunophenotypic characters of newly formed bile ducts (NFBs; ductular proliferations). **A.** Expresssion of beta-2-microglobulin by NFBs. **B, C.** Strong cytokeratin expression by NFBs (B: CAM 5.2; C: CK19). **D.** Focal cytoplasmic expression of vimentin by NFBs. Note that periportal hepatocytes in part express beta-2 microglobulin (A). ABC peroxidase stain. A: x 350; B: x 350; C: x 350: D: x 553

al., 1989), as have other accessory cells of the immune response; in particular, macrophages (Tobe, 1982; Tobe et al., 1982). It is, however, not yet clear if accumulation of APCs in PBC represents a phenomenon occurring throughout all stages of this disease, or rather represents a transient feature characteristic of initial phases of PBC. Theoretically, accessory cells accomplishing antigen presentation to effector lymphocytes may require their presence in the acquisition phase of immune reactions only.

In the present investigation, 58% of cases with early stage PBC exhibited cells with a dendritic morphology (dendritic cells, DCs) within the epithelia of interlobular and septal bile ducts. As reported in previous studies, these cells express HLA-DR and S-100 protein and are regarded as APCs (Hart and Fabre, 1981; Bardadin and Desmet, 1984; Van den Oord et al., 1986; Prickett et al., 1988; Demetris et al., 1989), and may adhere at sites of antigen delivery due to expression of ICAM-1 by bile duct cells (Adams et al., 1991). Interestingly, DCs were also stained with the monoclonal antibody KiMlp which is directed against antigen(s) expressed by cells of the monocyte/macrophage lineage (Radzun et al., 1991; Hansmann et al., 1992). Although dendritic accessory cells showed no reactivity with this antibody in its original description, we observed a reaction with DCs, probably due to the longer treatment of paraffin sections with protease, and the quality of staining was more closer to that obtained with anti-HLA-DR than that with anti-S-100 protein.

There is a correlation between the presence of DCs/APCs within bile duct epithelia and the expression of MHC products (Benacerraf, 1981) by the biliary epithelial cells. HLA-DR (MHC-class II) antigens, which serve as fundamental recognition and restriction elements for CD 4^+ T lymphocytes in immune responses (Biddison and Shaw, 1989), were expressed in bile duct cells in 10/12 samples of early stage PBC, and staining was observed in involved ducts only, both in infiltrated but not yet damaged, and in damaged bile ducts, and the most marked staining was seen in the latter, a finding not reported in previous studies (Ballardini et al., 1984; Van den Oord et al., 1986; Barbatis et al., 1987; Spengler et al., 1988). In one of the previous investigations, HLA-DR expression could not be demonstrated in stage I and stage II PBC (Shimizu et al., 1986). In contrast to other observations (Van den Oord et al., 1986), we noted a heterogeneous expression of MHC antigens in 4/12 cases where some ducts lacked HLA-DR expression or showed enhanced display of beta-2-microglobulin even though, in the same portal tracts, other duct epithelia were HLA-DR^+ and were surrounded by dense infiltrates. Interestingly, APCs were observed in some of the bile ducts which were HLA-DR^-, but generally they were found in higher numbers in HLA-DR^+ epithelia. These findings support, in accordance with a recent study (Nakanuma and Kono, 1991), the hypothesis that the biliary epithelial cells are at first altered in a still unknown way, express MHC antigens, and then fall victim to an immune attack. In contrast to former results (Nakanuma and Kono, 1991) we can show that there appears to be a correlation between ductal HLA-DR expression and intraepithelial accumulation of HLA-DR^+ APCs, and that both duct alterations and immune reactions are heterogeneous in space and time.

In contrast to bile ducts, APCs were not observed within the epithelial lining of ductular proliferations, which increase in amount as a function of disease progression. Ductular epithelia expressed beta-2-microglobulin similarly to interlobular bile ducts, but not HLA-DR. The lack of APC trapping in these structures may be related to an expression pattern of MHC products different from that of bile ducts, or to other differences which may be associated with lack of antigen expression. In this context, our finding that proliferated ductules express vimentin, in addition to typical cytokeratins, is of interest. Expression of vimentin in small bile ducts has been described in rats (Milani et al., 1989) and in some human hepatobiliary diseases (Nakanuma and Kono, 1992), as in our control group of non-PBC biliary diseases. Theoretically, vimentin expression may indicate ductular proliferation, remodelling, and/or a decrease in cellular differentiation (Shah and Gerber, 1989). During early embryonic development some cell types may show a transient expression of vimentin together with the constitutive tissue-specific intermediate filaments (Virtanen et al., 1985). Ductular proliferations consist of less differentiated cells, but the mechanisms of their production are still not well understood (Van Eyken et al., 1988; Uchikosi et al., 1992).

In late stage PBC, APCs are predominantly located in peripheral parts of portal tracts and within piecemeal necroses, in addition to previously described HLA-DR^+ spindle cells (Barbatis et al., 1981). This distribution pattern of APCs may have several explanations. Firstly, their accumulation at the parenchymal border, together with KiMlp$^+$ macrophages, may represent a reaction to lobular damage secondary to bile duct destruction, i.e. not directly linked to an autoimmune mechanism. It is the current view that late changes in PBC are most likely related to the effects of chronic obstructive cholestasis on hepatocytes and ducts/ductules, with subsequent biliary piecemeal necrosis and formation of fibrous septa (Nakanuma et al., 1990; Nakanuma, 1991). Cholestasis leading to cell damage also appears to exert an influence on HLA expression. Normal hepatocytes express few class I and no class II molecules (Franco et al., 1988; Bumgardner et al., 1989, 1990; Lobo-Yeo et al., 1990; Lindor, 1992), but it has been shown that hepatocytes can stimulate allospecific cytolytic T lymphocytes (So et al., 1987). Increased class I expression has been noted in several inflammatory liver diseases (Nagafuchi et al., 1985; Fukusato et al., 1986; So et al., 1987; Steinhoff et al., 1988) and here may be related to cholestasis (Innes et al., 1988; Arvieux et al., 1990; Calmus et al., 1990, 1992; Hillaire et al., 1991; Beuers et al., 1992; Lindor, 1992). Class I molecule expression by hepatocytes, as

seen in the present study, and induced by cholestasis may be of importance insofar as it has been shown that cells expressing MHC I are tolerogenic rather than immunogenic (Goeken, 1984; Baird et al., 1988; Arvieux et al., 1993). Thus, immunogenicity of hepatic parenchyma may be modified in cases of cholestasis (PBC included), and class I molecule expression could favour a state of tolerance (Arvieux et al., 1993). On the other hand, it is worth noting that, in the present study, class I expression by hepatocytes was seen in areas adjacent to piecemeal necrosis only, i.e. at sites where APCs had also accumulated. We assume that, in late stage PBC, autoimmune reactions may also take place at the lobular periphery, inducing the morphological pattern of chronic active hepatitis, which is a well-known change in PBC.

In conclusion, the present results indicate that: 1) APC distribution in PBC may change as a function of time and stage of disease; 2) ductules and their proliferations may not represent a target of immune attack, because no APC accumulation and no HLA-DR expression is found in these structures, and this may be related to an immature phenotype with vimentin expression; and 3) HLA expression in bile ducts appears to be heterogeneous, representing one mechanism why bile duct lesions in PBC do not occur in a synchronous way.

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References

Adams D.H., Hübscher S.G. and Shaw J. (1991). Increased expression of ICAM-1 in bile ducts in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 14, 426-432.

Arvieux C., Calmus Y., Gane P., Rouger P., Nordlinger B. and Poupon R. (1990). Quantitation and modulation of major histocompatibility complex (MHC) expression: The effect of cholestasis and immunosuppressive drugs. J. Hepatol. 11, S2.

Arvieux C., Calmus Y., Gane P., Legendre C., Mariani P., Delelo R., Poupon R. and Nordlinger B. (1993). Immunogenicity of rat hepatocytes in vivo: effect of cholestasis-induced changes in major histocompatibility complex expression. J. Hepatol. 18, 335-341.

Baird M.A., Munn S.R. and Heslop B.F. (1988). Immunosuppressive activity of class I antigens in the absence of antigen-presenting cells that are MHC compatible with the host. Transplantation 46, 105-108.

Ballardini G., Mirakian R., Bianchi F.B., Pisi E., Doniach D. and Bottazo G.F. (1984). Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. Lancet 2, 10011-10013.

Barbatis C., Woods J. and Morton J.A. (1981). Immunocytochemical analysis of HLA (A,B,C) antigens in liver disease using a monoclonal antibody. Gut 22, 985-991.

Barbatis C., Kelly P., Grevenson J., Heryet A. and Mc Gee J.O.D. (1987). Immunocytochemical analysis of HLA class II (DR) antigens in liver disease in man. J. Clin. Pathol. 40, 879-884.

Bardadin K.A. and Desmet V.J. (1984). Interdigitating and dendritic reticulum cells in chronic active hepatitis. Histopathology 8, 657-667.

Benacerraf B. (1981). Role of MHC gene products in immune regulation. Science 212, 1229-1238.

Beuers U., Spengler U. and Kruis W. (1992). Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebocontrolled trial. Hepatology 16, 707-714.

Biddison W.E. and Shaw S. (1989). CD4 expression and function in HLA class II-specific T cells. Immunol. Rev. 109, 5-15.

Bumgardner G.L., Chen S. and Hoffman R. (1989). Afferent and efferent pathways in T cell responses to MHC class I^+, II-hepatocytes. Transplantation 47, 163-170.

Bumgardner G.L., Matas A.J. and Chen S. (1990). Comparison of in vivo and in vitro immune response to purified hepatocytes. Transplantation 49, 429-436.

Calmus Z., Gane P., Rouger P. and Poupon R. (1990). Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 11, 12-15.

Calmus Y., Arvieux C., Gane P., Boucher E., Nordlinger B., Rouger P. and Poupon R. (1992). Cholestasis induces major histocompatibility complex class I expression in hepatocytes. Gastroenterology 102, 1371-1377.

Demetris J.A., Sever C., Kakizoe S., Oguma S., Starzl E.T. and Jaffe R. (1989). S-100 protein-positive dendritic cells in primary biliary cirrhosis and other inflammatory liver diseases. Am. J. Pathol. 134, 741-747.

Franco A., Barnaba V., Natali P., Balsano C., Musaca A. and Balsano F. (1988). Expression of class I and class II major histocompatibility complex antigens on human hepatocytes. Hepatology 8, 449-454.

Fregeau D.R., Roche T.E., Davis P.A., Coppel R. and Gershwin M.E. (1990). Primary biliary cirrhosis. Inhibition of pyruvate dehydrogenase complex activity by autoantibodies specific for Elalpha, a non-lipoic acid containing mitochondrial enzyme. J. Immunol. 144, 1671-1676.

Freer S.M. (1984). A permanent wet-mount for fluorescent microscopy of surface stained lymphoid cells. J. Immunol. Methods 66, 187-188.

Fukusato T., Gerber M.A., Thung S.N., Ferrone S. and Schaffner F. (1986). Expression of HLA class I antigens on hepatocytes in liver disease. Am. J. Pathol. 123, 264-270.

Goeken N. (1984). Human suppressor cell induction in vitro: preferential activation by class I MHC antigen. J. Immunol. 132, 2291-2293.

Hansmann M.L., Kikuchi M., Wacker H.H., Radzun H.J., Nathwani B.N., Hesse K. and Parwaresch M.R. (1992). Immunohistochemical monitoring of plasmacytoid cells in lymph node sections of Kikuchi-Fujimoto disease by a new pan-macrophage antibody Ki-M1P. Hum. Pathol. 23, 676-680.

Hart D.N.J. and Fabre J.W. (1981). Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. J. Exp. Med. 154, 347-361.

Hillaire S., Calmus Y. and Boucher E. (1991). Bile acids modulate hepatocyte HLA class I expression in human hepatocytes by a transcriptional mechanism (Abstract). Hepatology 14, A99.

Hsu S.M., Raine L. and Fanger H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled (PAP) procedures. J. Histochem. Cytochem. 29, 577-580.

Innes G.K., Nagafuchi Y., Fuller B.J. and Hobbs K.E.F. (1988).

Increased expression of major histocompatibility antigens in the liver as a result of cholestasis. Transplantation 45, 749-752.

James S.P., Hoofnagle J.H., Strober W. and Jones E.A. (1983). Primary biliary cirrhosis: A model autoimmune disease. Ann. Intern. Med. 99, 500-512.

Järvinen M. and Rinne A. (1983). The use of polyvinyl acetate glue to prevent detachment of tissue sections in immunohistochemistry. Acta Histochem. 72, 251-252.

Johnson D.A., Gautsch J.W., Sportsman J.R. and Elder J.H. (1984). Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal Tech. 1, 3.

King P.D. and Katz D.R. (1990). Mechanisms of dendritic cell function. Immunology Today II, 6, 206-211.

Lindor K. (1992). What is the role of major histocompatibility complex expression in cholestasis? Gastroenterology 102, 1429-1430.

Lobo-Yeo A., Senaldi G., Portman B., Mowat A.P., Mieli-Vergani G. and Vergani D. (1990). Class I and class II major histocompatibility complex antigen expression on hepatocytes: A study in children with liver disease. Hepatology 12, 224-232.

McKenzie J.L., Prickett T.C.R. and Hart D.N.J. (1989). Human dendritic cells stimulate allogeneic T cells in the absence of IL-I. Immunology 67, 290-297.

Milani S., Herbst H., Schuppan D., Niedobitek G., Kim K.Y. and Stein H. (1989). Vimentin expression of newly formed rat bile duct epithelial cells in secondary biliary fibrosis. Virchows Archiv. (A) 415, 237-242.

Nagafuchi Y., Thomas H.C., Hobbs K.E.F. and Scheuer P.J. (1985). Expression of beta-2-microglobulin on hepatocytes after liver transplantation. Lancet 1, 551-554.

Nakanuma Y. (1991). Pathology of septum formation in primary biliary cirrhosis: a histological study in the non-cirrhotic stage. Virchows Archiv. (A) 419, 381-387.

Nakanuma Y. and Kono N. (1991). Expression of HLA-DR antigens on interlobular bile ducts in primary biliary cirrhosis and other hepatobiliary diseases: An immunohistochemical study. Hum. Pathol. 22, 431-436.

Nakanuma Y. and Kono N. (1992). Expression of vimentin in proliferating and damaged bile ductules and interlobular bile ducts in nonneoplastic hepatobiliary diseases. Mod. Pathol. 5, 550-554.

Nakanuma Y., Saito K. and Unoura M. (1990). Semiquantitative assessment of cholestasis and lymphocytic piecemeal necrosis in primary biliary cirrhosis: a histologic and immunohistochemical study. J. Clin. Gastroenterol. 12, 357-362.

Prickett T.C., McKenzie J.L. and Hart D.N. (1988). Characterization of interstitial dendritic cell in human liver. Transplantation 46, 754-761.

Radzun H.J., Hansmann M.L., Heidebrecht H.J., Bödewadt-Radzun S., Wacker H.H., Kreipe H., Lumbeck H., Hernandez C., Kuhn C. and Parwaresh M.R. (1991). Detection of a monocyte/macrophage differentiation antigen in routinely processes paraffin embedded tissues by monoclonal antibody Ki-MI p. Lab. Invest. 65, 306-315.

Ruchti C.H., Gerber H.A. and Schaffner T.H. (1984). Factor VIII-related antigen in malignant hemangioendothelioma of the thyroid: additional evidence for the endothelial origin of this tumor. Am. J. Clin. Pathol. 82, 474-480.

Scheuer P.J. (1967). Primary biliary cirrhosis. Proc. R. Soc. Med. 60, 1257-1260.

Shah K.D. and Gerber M.A. (1989). Development of intrahepatic bile ducts in humans. Arch. Pathol. Lab. Med. 113, 1135-1138.

Sherlock S. (1989). Primary biliary cirrhosis. In: Diseases of the liver and biliary system. Sherlock S. (ed). Blackwell Scientific Publications. Oxford. pp 273-288.

Shimizu M., Yuh K. and Ichihara I. (1986). Immunohistochemical characterization of inflammatory infiltrates at the site of bile duct injury in primary biliary cirrhosis. Liver 6, 1-6.

So S.K.S., Platt J.L., Ascher N.L. and Snover D.C. (1987). Increased expression of class I major histocompatibility complex antigen on hepatocytes in rejecting human liver allografts. Transplantation 43, 79-85.

So S.K.S., Wilkes L.M., Platt J.L. and Simmons R.L. (1987). Purified hepatocytes can stimulate allospecific cytolytic T lymphocytes in a mixed lymphocyte-hepatocyte culture. Transplant Proc. 19, 251-252.

Spengler U., Pape G.R., Hoffman R.M., Johnson J.P., Eisenburg J. and Paumgartner-Riethmüller G. (1988). Differential expression of MHC class II subregion product on bile duct epithelial cells and hepatocytes in patients with primary biliary cirrhosis. Hepatology 8, 459-462.

Steinhoff W., Wonigeit K. and Pichlmayr R. (1988). Analysis of sequential changes in major histocompatibility complex expression in human liver grafts after transplantation. Transplantation 45, 394-401.

Surh C.D., Coppel R. and Gershwin M.E. (1990). Structural requirements for autoreactivity of human pyruvate dehydrogenase E2, the major autoantigen of primary biliary cirrhosis. Implication for a conformational autoepitope. J. Immunol. 144, 3367-3374.

Tobe K. (1982). Electron microscopy of liver lesions in primary biliary cirrhosis. II. A bile duct with chronic non-suppurative destructive cholangitis. Acta Pathol. Jpn. 32, 345-357.

Tobe K., Endo H., Nakashima H. and Kobayashi T. (1982). Langerhans cells among bile duct epithelial cells in chronic liver disease. Acta. Pathol. Jpn. 32, 93-99.

Uchikosi Y., Kajikazawa N., Tanaka Y., Asakura H. (1992). Appearance of hepatocyte-like cells in the interlobular bile ducts of human liver in various liver disease stages. Hepatology 16, 199-205.

Van den Oord H.H., Sciot R. and Desmet V. (1986). Expression of MHC products by normal and abnormal bile duct epithelium. Hepatology 3, 310-317.

Van de Water J., Fregeau D., Davis P., Ansari A., Danner D., Leung P., Coppel R. and Gershwin M.E. (1988). Autoantibodies of primary biliary cirrhosis (PBC) recognize dihydrolipoamide acetyltransferase and inhibit enzyme function. J. Immunol. 141, 2321-2324.

Van Eyken P., Sciot R., Callea F., Van der Steen K., Moerman P. and Desmet V.J. (1988). The development of the intrahepatic bile ducts in man: A keratin immunohistochemical study. Hepatology 8, 1586-1595.

Virtanen I., Miettinen M. and Lehto V.P. (1985). Diagnostic application of monoclonal antibodies to intermediate filaments. Ann. N.Y. Acad. Sci. 455, 635-648.

Yeaman S.J., Danner D., Mutimer D.J., Fussey S.P.M., James O.F.W. and Basendine M.S. (1988). Primary biliary cirrhosis: Identification of two major M2 mitochondrial autoantigens. Lancet 1, 1067-1069.

Yoshida T., Bonkovsky H., Ansari A. and Danner A. (1990). Antibodies against mitochondrial dehydrogenase complexes in primary biliary cirrhosis. Gastroenterology 99, 187-194.

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