Virchows Arch. A Path. Anat. and Histol. 373, 143–160 (1977)

Virchows Archiv A

Histopathological Features in Mixed Types of Chronic Aggressive Hepatitis and Primary Biliary Cirrhosis

Correlations of Liver Histology with Mitochondrial Antibodies of Different Specificity

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Summary. A histopathological study was carried out on 27 patients with chronic inflammatory liver disease and clinical and/or biochemical evidence of cholestasis who had either mitochondrial antibodies against mitochondrial antigen fractions of 1.19 density ("PBC antigen"; 14 cases) or of 1.13 density ("CAH-PBC mixed-type antigen"; 13 cases). For comparison, the liver biopsies of 17 patients with chronic-aggressive hepatitis (CAH) and antinuclear and/or anti-smooth muscle antibodies but without cholestasis and mitochondrial antibodies, were evaluated. The 14 patients with mitochondrial antibodies against the PBC antigen showed the typical histological features of primary biliary cirrhosis (PBC). The 13 patients with mitochondrial antibodies against the CAH-PBC mixed-type antigen had heterogenous liver alterations. In 11 cases highly active CAH and/or active postnecrotic cirrhosis (AC) were found both with augmented ductular proliferation. Some of these cases showed distinct criteria of PBC as early bile duct lesions or absence of regular bile ducts. The liver histology of one case corresponded to classical PBC; another case to chronic persistent hepatitis. The CAH-patients without cholestasis and mitochondrial antibodies only occasionally showed bile duct proliferation. In conclusion, a high correlation was found between mitochondrial antibodies against the CAH-PBC mixed-type antigen and highly active CAH or early AC with augmented ductular proliferation. This represents an overlapping of CAH and PBC. In contrast, the cases with antibodies reacting to the PBC antigen showed the slowly progressive liver changes of typical PBC.

Key words: Chronic aggressive hepatitis – Primary biliary cirrhosis – Mixed types – Mitochondrial antibody specificity.

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Introduction

Primary biliary cirrhosis (PBC) is of increasing importance in the differential diagnosis of chronic inflammatory liver disease. Its frequency seems to have increased in the last 15 years (Ahrens et al., 1950; Sherlock, 1959; Hoffbauer, 1960; Foulk et al., 1964; Brunner et al., 1972; Sherlock and Scheuer, 1973; Schmidt et al., 1976). As to the morphogenesis of PBC, it was recognized that the initial damage involves the bile ducts resulting in chronic non-suppurative destructive cholangitis (Rubin et al., 1965) which progresses to cirrhosis in a sequenced course (Hanot, 1876; Scheuer, 1967). Recently, the laparoscopic appearance of the liver in PBC was classified, showing good correlation with the staging of PBC at light microscopical level (Lindner et al., 1975). In addition to liver histology the presence of mitochondrial antibodies (Walker et al., 1965) directed against parts of the inner membranes of mitochondria (Berg et al., 1969; Bianchi et al., 1973), are of particular importance in establishing the diagnosis, because they are detected in over 80% to 90% of patients (Doniach, 1970; Berg and Doniach, 1972; Brunner et al., 1972; Sherlock and Scheuer, 1973; Pisi et al., 1975). However, they may also be rarely found in chronic aggressive hepatitis (CAH), especially in the cholestatic variant which shares many clinical, histological and immunological features with PBC (Jones and Tisdale, 1963; Hadziyannis et al., 1970; Sherlock, 1970; Berg and Doniach, 1972; Berg et al., 1973; Pisi et al., 1975; Ruckstuhl et al., 1971). Furthermore, mitochondrial antibodies have been detected in patients with pseudo-lupus erythematosus (Maas and Schubothe, 1973; Berg et al., 1975; Grob et al., 1975). In order to overcome these diagnostic problems the antigen specificity of the mitochondrial antibodies has been tested with different mitochondrial antigen fractions, separated by moving-zone centrifugation, and shown to be heterogenous (Berg et al., 1973; Berg et al., 1975). An antigen fraction (1.10 density) reacted specifically with mitochondrial antibodies from patients with a pseudolupus erythematosus syndrome and was called "PLE antigen". Another fraction (density 1.19) reacted specifically with mitochondrial antibodies from patients diagnosed as primary biliary cirrhosis which was called "PBC antigen". In a third group of patients who frequently showed features of both CAH and PBC mitochondrial antibodies in their serum reacted specifically with an antigen fraction of a density 1.13, and this was called the "CAH-PBC mixed-type antigen" (Berg and Binder, 1976; Berg et al., 1976).

Based on this recent immunological differentiation, the present work examines whether patients with mitochondrial antibodies against the CAH-PBC mixed-type antigen (group II) also differ in their liver histopathology from patients with mitochondrial antibodies against the PBC antigen (group I) and from those with CAH but without mitochondrial antibodies and cholestasis (group III).

Material and Methods

Mitochondrial antibodies were demonstrated in 27 patients by an indirect immunofluorescent technique using various human and animal organs as substrate, and by a qualitative complement fixation method using rat kidney mitochondria (for details see Berg et al., 1975). In order to obtain subfrac-

Table 1. Demonstration of antinuclear antibodies (ANA) and smooth muscle antibodies (SMA) in patients with antimitochondrial antibodies (AMA) directed either against the "PBC-antigen" (group I) or the "CAH-PBC mixed-type antigen" (group II)

Case No.	Initials	AMA to PBC antigen	AMA to CAH-PBC antigen	ANA	SMA
1.	E., G.	+	−	−	+
2.	A., A.	+	−	−	−
3.	F., E.	+	−	−	−
4.	L., F.	+	−	−	−
5.	M., E.	+	−	−	−
6.	H., A.	+	−	(+)	−
7.	P., H.	+	−	−	−
8.	G., L.	+	−	−	−
9.	M., J.	+	−	−	−
10.	S., E.	+	−	−	+
11.	R., L.	+	−	−	−
12.	J., U.	+	−	−	−
13.	B., H.	+	−	+	−
14.	L., E.	+	−	−	−
15.	S., E.	−	+	−	−
16.	B., H.	−	+	−	++
17.	S., A.	−	+	−	++
18.	P., M.	−	+	+	+
19.	G., H.	−	+	−	+
20.	H., M.	−	+	+	+
21.	B., L.	−	+	+	+
22.	T., R.	−	+	−	+
23.	Bu., H.	−	+	−	++
24.	S., C.	−	+	++	++
25.	W., P.	−	+	+	+
26.	K., L.	−	+	−	−
27.	C., A.	−	+	−	−

tions of mitochondrial antigens the supernatant of a cytoplasmic extract from rat kidneys, containing small fragments of mitochondria, was separated by a combined saccharose gradient isopycnic centrifugation (for details see Berg et al., 1975; Berg and Binder, 1976). The gradient fraction of 1.19 density reacted specifically with sera from 14 patients (Table 1; group I; "PBC antigen") in the complement fixation test. Sera of 13 other patients (Table 1; group II) fixed complement with a gradient fraction of 1.13 density ("CAH-PBC mixed-type antigen"). The clinical, biochemical and detailed immunoserological data of the patients with mitochondrial antibodies will be summarized in a future paper. Seventeen other patients, suffering from non-cholestatic chronic inflammation of the liver, were also histologically examined (group III). They had no mitochondrial antibodies, but at one time or another showed antibodies directed against nuclei, smooth muscle and/or vascular epithelium. Three were HB_s-Ag positive.

Each of the 27 patients underwent, at least, one liver biopsy. Fourteen of these had follow-up biopsies (Table 2). Altogether a total of 54 liver specimens was examined (51 needle biopsy specimens; 3 wedge biopsies). In addition, there was autopsy material available for examination in 3 cases. – Forty four liver biopsies were available from the 17 patients without mitochondrial antibodies.

Histological examination of liver biopsies was made by two observers without knowledge of detailed clinical findings. The liver tissue was fixed in 4% formaldehyde solution. Sections were

Table 2. Age, time interval under observation, and number of biopsies (group I and II)

Case No.	Initials	Sex	Age at first biopsy (ys)	Duration until final biopsy	Number of biopsies
1.	E., G.	F	63	11 months	2
2.	A., A.	F	45	–	1
3.	F., E.	F	56	1 month	2
4.	L., F.	M	63	–	2
5.	M., E.	F	48	2 years	4
6.	H., A.	F	52	–	1
7.	P., H.	F	47	1 year	3
8.	G., L.	F	53	3 years	4
9.	M., J.	F	57	5 years	4
10.	S., E.	F	61	3 years[a]	1
11.	R., L.	F	65	4 years[a]	1
12.	J., U.	F	61	1 year	2
13.	B., H.	F	65	–	1
14.	L., E.	F	63	–	1
15.	S., E.	F	73	–	1
16.	B., H.	F	51	–	2
17.	S., A.	F	79	–	1
18.	P., M.	F	65	–	1
19.	G., H.	F	63	–	2
20.	H., M.	F	80	–	1
21.	B., L.	F	73	1 year	2
22.	T., R.	F	42	–	1
23.	Bu., H.	F	73	4 years	5
24.	S., C.	F	66	4 years	5
25.	W., P.	F	65	5 years[a]	2
26.	K., L.	F	56	–	1
27.	C., A.	F	50	–	1

[a] In these cases additional autopsy material was examined

stained with hematoxylin-eosin, periodic acid Schiff (PAS), Masson-Goldner's technique for connective tissue, and with Prussia blue.

The histological findings were grouped under the following headings (Tables 3 and 4): (1) Intact portal tracts (+ = one or two; + + = three to five; + + + = more than five) without inflammatory infiltration and with normal bile ducts; (2) chronic periportal inflammation (+ = weak; + + = moderate; + + + = severe) with periportal erosion of the liver parenchyma but without bile duct damage or ductular proliferation; (3) chronic non-suppurative destructive cholangitis (+ = one floride duct lesion; + + = one or two floride duct lesions associated with parabiliary granuloma; + + + = more than two floride duct lesions and parabiliary granulomas) being defined as the initial stage of PBC (PBC I) (Rubin et al., 1965); (4) ductular proliferations (+ = few to + + + = many) extending into the periportal parenchyma associated with distinct reduction of intact bile ducts, portal fibrosis and inflammation (PBC II); (5) sepetal or stellate scars (+ = single to + + + = many) almost devoid of intact bile ducts or ductular proliferates, and with minimal inflammatory infiltration (PBC III); (6) net-like cirrhosis to nodular cirrhosis, representing the last stage of PBC (PBC IV), with absence of ductular proliferates and interlobular bile ducts, as well as with a highly reduced number of septal ducts; (7) active postnecrotic cirrhosis (+ = moderate; + + = marked; + + + = severe periportal erosion) characterized by inflammatory infiltration, bridging fibrosis, and augmented bile duct proliferation; (8) cholestasis (+ = few to + + + = many bile thrombi).

Table 3. Main histological findings in liver biopsies from 17 patients with mitochondral antibodies to the "PBC antigen" (group I). The severity and extent of the findings are graded in + to + + + scale (for further details see text)

Case No.	Initials	Year	Normal portal tracts with intact bile ducts	Chronic portal and periportal inflammation	Bile duct damage and parabiliary granulomas	Bile duct proliferations devoid of intact bile ducts	Septal scars devoid of intact bile ducts	Cirrhosis devoid of ductular structures	Active postnecrotic cirrhosis with ductular prolifer.	Cholestasis	Histological diagnosis[a]
1.	E., G.		–	–	+	–	–	–	–	–	PBC I
2.	A., A.		–	–	+ +	–	–	–	–	–	PBC I
3.	F., E.		–	–	+ + +	–	–	–	–	–	PBC I
4.	L., F.		–	–	+ + +	–	–	–	–	–	PBC I
5.	M., E.	1973	–	–	+	–	–	–	–	–	PBC I
		1974	+	+	–	–	–	–	–	–	
		1975	+ (fatty liver)	–	–	–	–	–	–	–	
6.	H., A.		–	–	+	+	–	–	–	–	PBC I–II
7.	P., H.	1975	–	–	–	+ +	+	–	–	–	PBC I→III
		1976	–	–	–	+	+ +	–	–	–	
8.	G., L.	1972	–	–	–	+	–	–	–	–	PBC II→III
		1973	–	–	–	+ +	–	–	–	–	
		1974	–	–	–	+	+	–	–	–	
		1975	–	–	–	+	+ +	–	–	–	
9.	M., J.	1969	–	–	–	+ +	+	–	–	+	PBC II→IV
		1973	–	–	–	+	+ +	→+	–	+ + +	
		1974	–	–	–	+	–	+	–	+ + +	
10.	S., E.	1967	–	–		+	+ + +	–	–	–	PBC III→IV
		1970[b]	–	–	–	–	–	+	–	+ + +	
11.	R., L.	1971	–	–	–	+	+ +	–	–	–	PBC III→IV
		1975[b]	–	–	–	–	–	+	–	+ + +	
12.	J., U.		–	–	–	+ +	+ + +	→+	–	+ +	PBC II–IV
13.	B., H.		–	–	–	–	–	+	–	+ + +	PBC IV
14.	L., E.		–	–	–	+	–	+	–	+ + +	PBC IV

[a] PBC = primary biliary cirrhosis; AC = active postnecrotic cirrhosis with augmented ductular proliferation; arrow means transition to

[b] Autopsy material

Results

1. Liver Histopathology of Patients with Chronic Inflammatory and Cholestatic Liver Disease and Mitochondrial Antibodies against the PBC Antigen (Group I)

The most important findings are summarized in Table 3. All 14 cases revealed liver changes chracteristic of PBC according to Rubin et al. (1965), Scheuer (1967) and Schaffner (1975): 6 cases showed PBC I, 3 cases PBC II, 2 cases PBC III and 3 cases PBC IV in their first biopsy.

In stage I almost all interlobular and some septal bile ducts were affected by severe inflammation, which enlarged the portal tracts (Fig. 1). The epithelium of the affected bile ducts was swollen or apparently necrotic or ruptured. The intense inflammatory infiltration around the ducts chiefly consisted of lymphocytes, plasma cells, and macrophages, the cytoplasm of which was often PAS-positive. Moreover, the infiltrate contained aggregates of epitheloid cells forming poorly defined parabiliary granulomas (Fig. 1). Giant cells or lymph follicles with germinal centres were not seen. Occasionally some of the bile ducts were seen to be replaced by fibrous tissue. In the small portal tracts, which were consistantly infiltrated by mononuclear cells, the bile ducts might be damaged

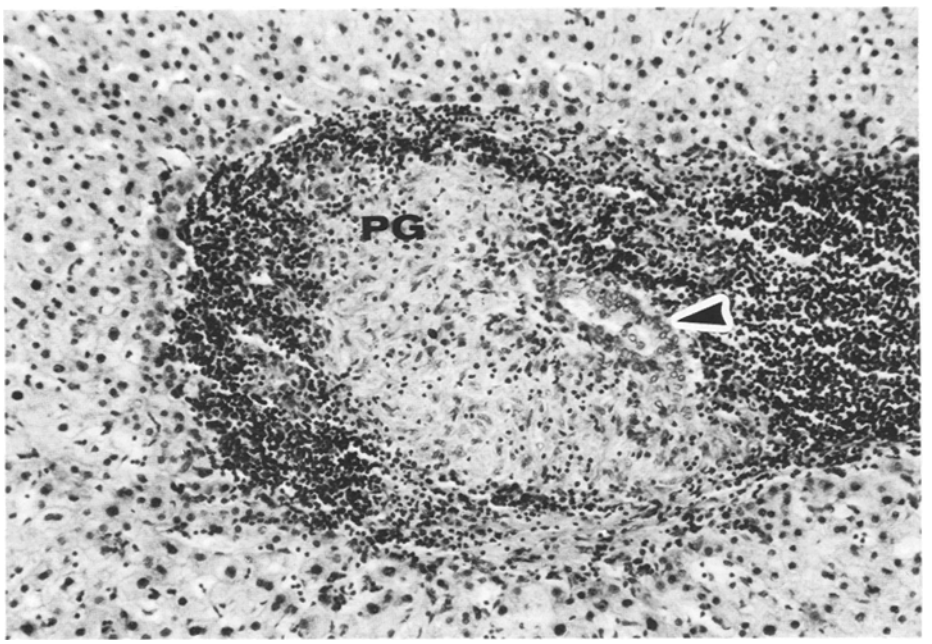


Fig. 1. PBC stage I in a patient with mitochondrial antibodies to the "PBC antigen" (case 4): the portal tract is enlarged by a parabiliary granuloma (*PG*) composed of epitheloid macrophages and surrounded by a small rim of lymphoid cells. The parabiliary granuloma is in close contact with a damaged interlobular bile duct (arrow). The liver parenchyma is not affected by the inflammatory process. Hematoxylin-eosin. × 120

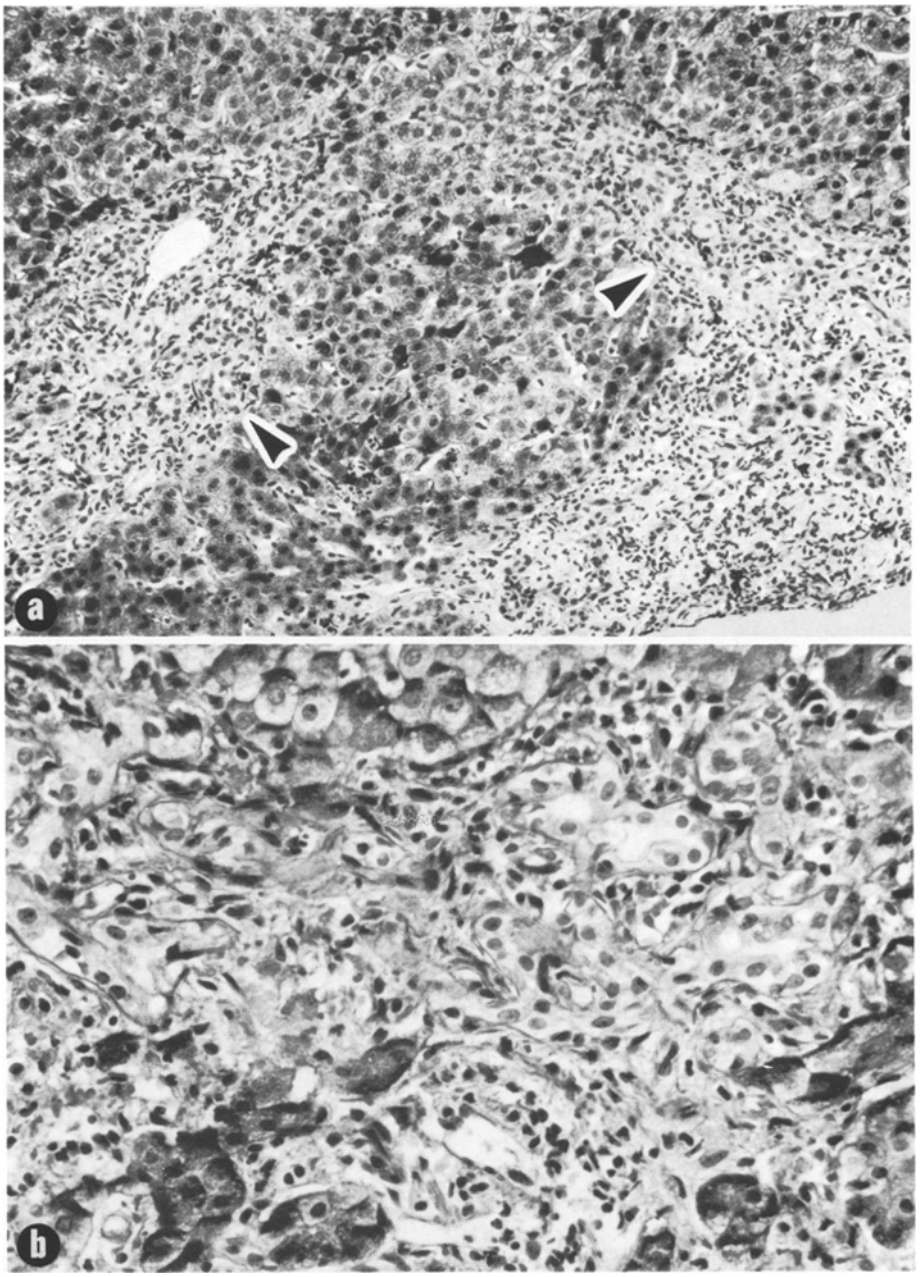


Fig. 2a and b. PBC stage II in a patient with mitochondrial antibodies to the "PBC antigen" (case 7): **a** Portal tracts showing fibrosis, diffuse round cell infiltration, slight periportal erosion of liver parenchyma, absence of intact bile ducts, and ductular proliferations (arrows). Masson-Goldner. ×120. **b** Ductular proliferations invading the parenchyma. Hematoxylin-eosin. ×300

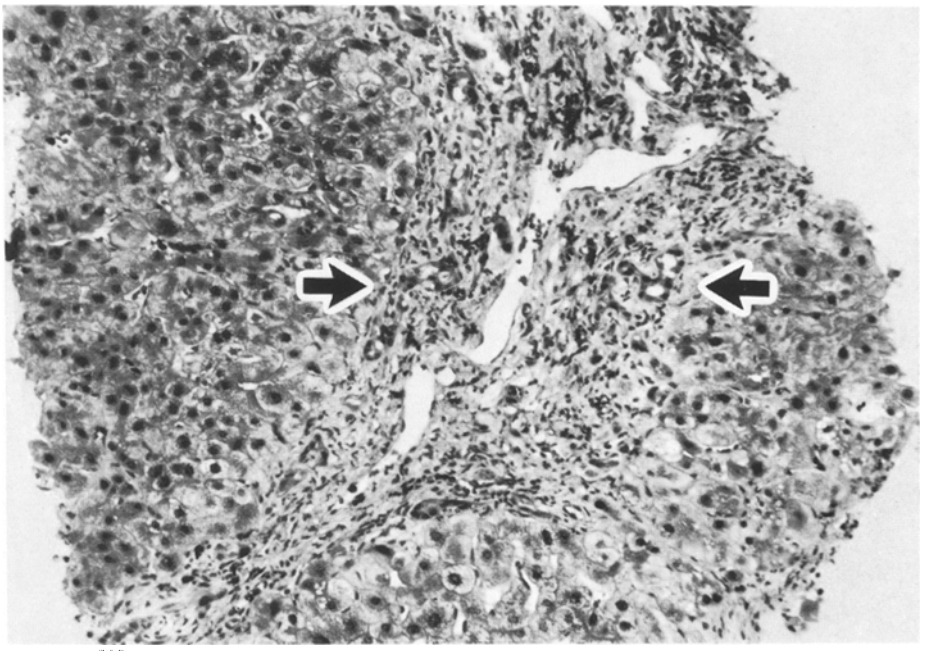


Fig. 3. PBC stage II–III in a patient with mitochondrial antibodies to the "PBC antigen" (case 9): septal scar devoid of intact bile ducts and dissecting the parenchyma. The fibrous septum still contains few ductular proliferations (arrows). Hematoxylin-eosin. × 120

or unaffected. The limiting plate of hepatocytes surrounding the portal tracts was intact, although scattered mononuclear cells were found within the parenchyma. The Kupffer cells appeared to be activated. Cholestasis was absent.

In stage II the portal tracts, which were expanded by fibrous tissue and round cell infiltrates, lacked normal bile ducts (Fig. 2a). They contained ductular proliferations, breaking through the limiting plates, with poorly defined lumens and flattened epithelial cells (Fig. 2b). Granulomas were notably absent. The inflammatory infiltrate often extended into the parenchyma. Necroses of hepatocytes of the limiting plate however, were rare. Cholestasis was not present.

In stage III septal scarring developed. This was associated with regression of the chronic inflammatory infiltrate and disappearance of the ductular proliferation (Fig. 3). Active inflammatory processes were not observed. Some cases showed transition to cirrhosis. Peripheral cholestasis was found in one case.

In stage IV irregular fibrous septa distorted the normal parenchymal architecture. The septa, which showed minimal inflammatory infiltrates, were small and devoid of ductular elements or normal bile ducts. Only some main septal ducts remained and appeared to be dilated. Apart from one case with early PBC IV, all other cases showed diffuse cholestasis. A typical micronodular appearance of cirrhosis was observed in fully developed cirrhotic stages.

Follow-up biopsies revelaed progression of the liver alterations in 5 cases. In one case the combined criteria of stages I and II were found. The first biopsy in case 5 showed PBC I, which disappeared from the follow-up biopsies.

Table 4. Main histological findings in liver biopsies from 17 patients with mitochondrial antibodies to the "CAH-PBC mixed-type antigen" (group II). The severity and extent of the findings are graded in + to +++ scale (for further details see text)

Case No.	Initials	Year	Normal portal tracts with intact bile ducts	Chronic portal and periportal inflammation	Bile duct damage and parabiliary granulomas	Bile duct proliferations devoid of intact bile ducts	Septal scars devoid of intact bile ducts	Cirrhosis devoid of ductular structures	Active postnecrotic cirrhosis with ductular prolifer.	Cholestasis	Histological diagnosis[a]
15.	S., E.		++	+	–	–	–	–	–	–	CPH
16.	B., H.		–	+++	–	++	–	–	→+	–	CAH-AC
17.	S., A.		–	+	–	–	–	–	→+	–	CAH-AC
18.	P., M.		–	–	–	–	–	–	++	+++	AC
19.	G., H.		–	–	–	+	–	–	+++	–	AC
20.	H., M.		–	+	–	+	++	–	–	–	CAH-PBC
21.	B., L.	1971	–	+	++	+	+	–	–	+	CAH-PBC
		1972	–	+	+	+	++	–	–	+	
22.	T., R.		–	++	–	+	+	–	–	+++	CAH-PBC
23.	Bu., H.	1972	–	++	++	–	–	–	–	–	CAH-PBC
		1976	–	+	+	+	–	–	++	–	→AC-PBC
24.	S., C.	1972	–	++	–	+	–	–	–	–	CAH-PBC
		1973	–	++	–	++	–	–	–	–	→AC-PBC
		1974	–	+++	–	++	–	–	–	–	
		1976	–	+++	–	++	–	–	→+	++	
25.	W., P.	1970	–	+++	+	++	+	–	–	+	CAH-PBC
		1975[b]	–	–	–	–	–	+	–	+++	→PBC IV
26.	K., L.		–	++	++	++	–	–	+++	+	AC-PBC
27.	C., A.		–	–	–	+	++	–	–	–	PBC II–III

[a] CPH=chronic persistent hepatitis; CAH=chronic aggressive hepatitis; AC=active postnecrotic cirrhosis with augmented ductular proliferation; PBC=primary biliary cirrhosis; arrow means transition to

[b] Autopsy material

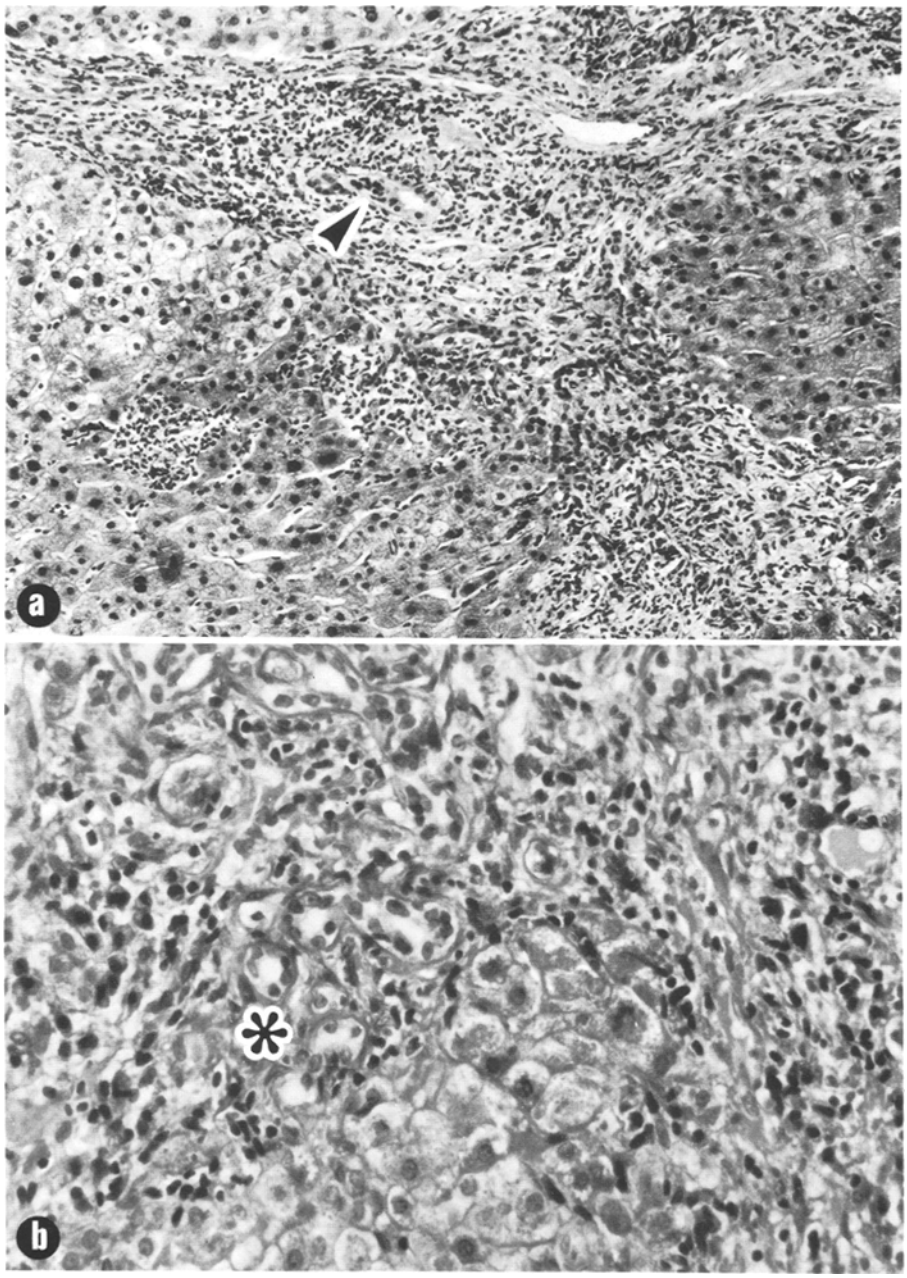
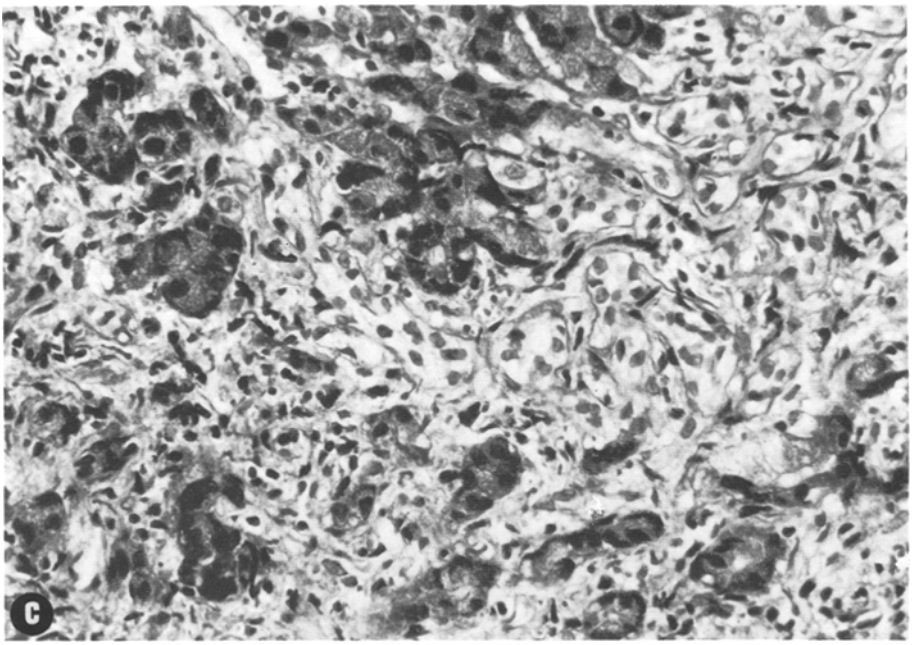


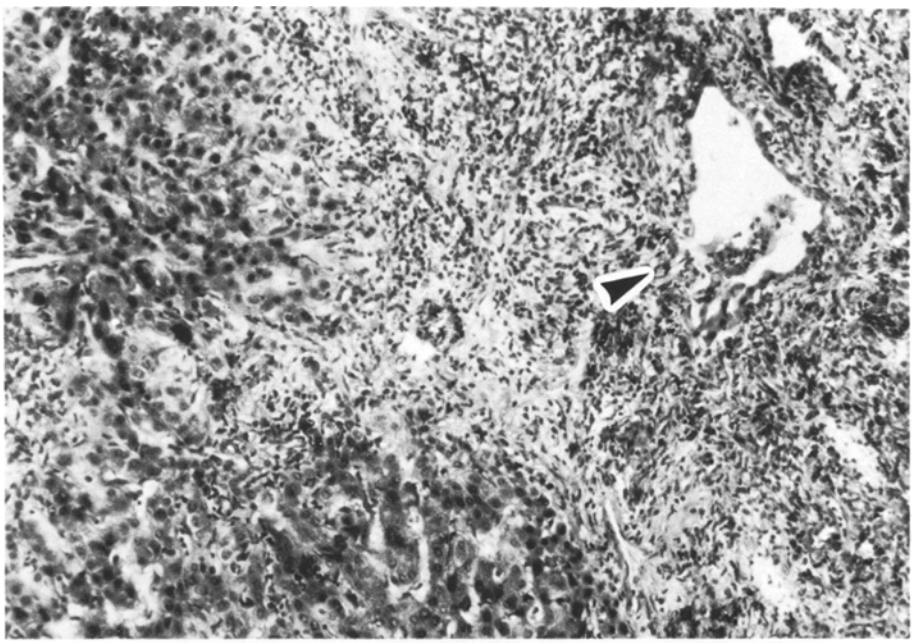
Fig. 4a–c. Chronic aggressive hepatitis (CAH) associated with PBC criteria in patients with antibodies to the "CAH-PBC mixed type antigen" **a** (case 21) Enlarged portal tract showing damaged bile duct (arrow) and marked lymphoid cell infiltration encroaching the parenchyma. Hematoxylin-eosin. ×120. **b** (case 16) Periportal erosion of the limiting plate of hepatocytes associated with ductular proliferation in the periportal area (asterisk). Hematoxylin-eosin. ×300. **c** (case 24) Marked ductular proliferates, indistinguishable from those seen in PBC stage II. PAS. ×300



2. Liver Histopathology of Patients with Chronic Inflammatory and Cholestatic Liver Disease and Mitochondrial Antibodies against the CAH-PBC Mixed-Type Antigen (Group II)

The most important findings are summarized in Table 4. The liver biopsies of the 13 cases showed heterogenous changes. In 7 cases criteria of CAH and PBC (CAH-PBC) or of active postnecrotic cirrhosis (AC) and PBC (AC-PBC) were observed. In 4 other cases severe CAH with transition to AC or complete AC were present. One case showed a chronic hepatitis resembling chronic persistent hepatitis. Only one case had criteria of typical PBC.

All 7 cases with criteria of CAH/AC on the one hand and of PBC on the other hand revealed a highly active periportal inflammation in association with considerable bile duct proliferation (Fig. 4). The periportal inflammation eroded the limiting plate of hepatocytes (Fig. 4b). Furthermore, portal-central or portal-portal inflammatory connective tissue bridges were developed. When the latter changes were observed, an active postnecrotic cirrhosis (AC) was diagnosed (Fig. 5). The inflammatory process also involved the biliary system, affecting the bile ducts to a variable extent. The changes seen consisted of lesions of the epithelium, formation of parabiliary granulomata, and, in particular, ductular proliferation in association with loss of normal duct structures (Fig. 4b and c). Focal scars without bile ducts were also present. In 4 cases with CAH-PBC or AC-PBC all bile duct changes mentioned above concurrently occurred. In 2 cases with CAH-PBC, only ductular proliferation and loss of normal bile ducts were obvious. Five of the 7 cases had cholestasis.



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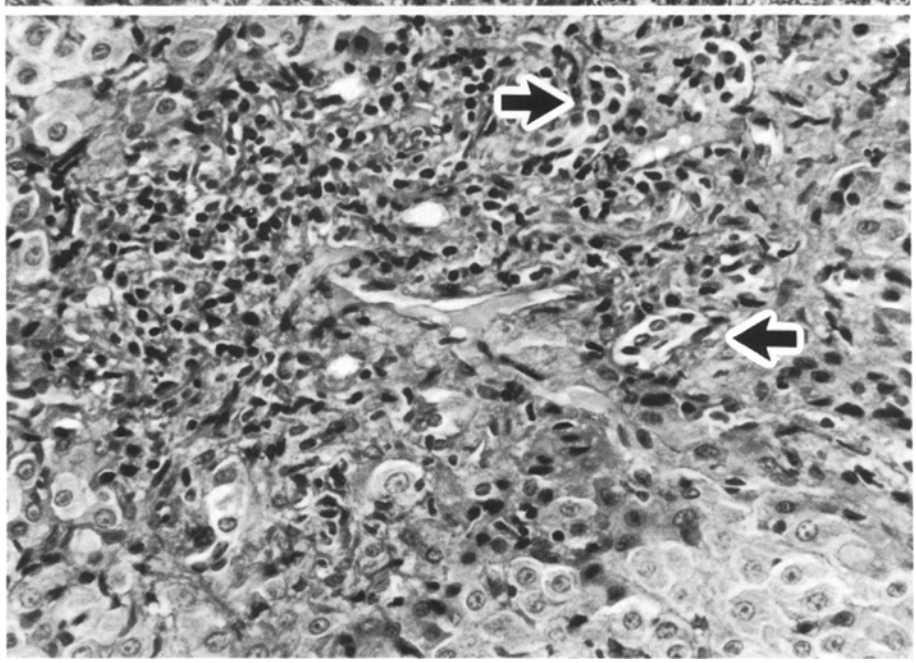

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Fig. 5. Active postnecrotic cirrhosis associated with PBC criteria in a patient with mitochondrial antibodies to the "CAH-PBC mixed-type antigen" (case 26): damaged large interlobular bile duct, and extensive inflammatory infiltration in the portal zone and the adjacent parenchyma. Masson-Goldner. ×120

Fig. 6. Highly active chronic aggressive hepatitis without cholestasis and mitochondrial antibodies: heavy periportal infiltration. Only single small bile duct proliferations are seen at the periphery of the portal area (arrows). Hematoxylin-eosin. ×300

The 4 cases which were classed as CAH or AC also showed augmented bile duct proliferation, although some normal bile ducts remained. Thus these cases did not really fullfill the criteria for PBC II. One case had cholestasis; another showed the typical criteria of PBC II–III.

3. Liver Histopathology of Patients with Chronic Inflammatory Non-Cholestatic Liver Disease and Circulating Autoantibodies Other than Mitochondrial Antibodies (Group III)

Four cases had chronic persistent hepatitis. Six cases had CAH of moderate activity. Seven cases had CAH of severe activity, two of which already showed transition to cirrhosis. None of the 17 cases fullfilled the criteria of PBC I, II, III or IV. In 3 cases with highly active CAH, ductular proliferation was observed in addition to intact bile ducts. However, the extent of the ductular proliferation in the portal tracts was low, as compared with CAH-PBC mixed types or PBC II (Fig. 6). The ductular proliferations had lumina of varying width and, in general, had flattened epithelial cells. They were scattered in the portal areas. Cholestasis was absent in all cases.

Discussion

The histopathology of primary biliary cirrhosis (PBC) runs a sequenced course, starting with chronic non-suppurative destructive cholangitis and developing into cirrhosis (Hanot, 1876; Foulk et al., 1964; Rubin et al., 1965; Scheuer, 1967; Schaffner, 1975). In addition to liver histology, the demonstration of mitochondrial antibodies provides an important clue in the diagnosis of PBC (Walker et al., 1965; Sherlock and Scheuer, 1973). However, mitochondrial antibodies may also occur in other liver diseases, particularly in "lupoid" chronic aggressive hepatitis (CAH) with cholestasis, or in "cryptogenic" cirrhosis (Hadziyannis et al., 1970; Berg and Doniach, 1972; Pisi et al., 1975; Schaffner, 1975). Furthermore, they are found in pseudo-lupus erythematosus (PLE) (Maas and Schobothe, 1973; Berg et al., 1975; Grob et al., 1975). Recently Berg and his associates showed that mitochondrial antibodies are heterogenous and react with mitochondrial antigen fractions of different density (Berg et al., 1975; Berg and Binder, 1976; Berg et al., 1976). Their antigen fractions were obtained from cytoplasmic extracts by using a combined saccharose moving-zone centrifugation, and on this basis so-called PLE, PBC, and CAH-PBC mixed-type antigens have been distinguished (Berg et al., 1976).

Primary Biliary Cirrhosis

All 14 patients (13 women and 1 man) with chronic inflammatory liver disease, cholestasis, and mitochondrial antibodies against the PBC antigen had liver changes resembling those thought to be characteristic of PBC (Rubin et al.,

1965; Scheuer, 1967; Sherlock and Scheuer, 1973; Schaffner, 1975). The most conspicious findings of stage I were damage to the interlobular bile duct epithelium with parabiliary granulomas in the large portal tracts. The inflammatory infiltration, and occasionally the ductular destructive processes spread to the small portal tracts. This typical stage was present in one third of the cases and was easily isolated from the following stages. Another third of the patients was found to be in stage II or III. It was not possible to discriminate exactly between stage II and stage III, since ductular proliferation within the portal areas was often associated with septal scars devoid of ductular structures. The degree of this latter finding indicated progression of the disease. The change-over to the final stage – true cirrhosis – was also continuous, thus characteristics of stages II, III and IV appeared in the same biopsy in some cases. The early cirrhotic stage was characterized by a net-like fibrosis dissecting the liver parenchyma, while in late cirrhosis, as is present in autopsy cases, a typical micronodular pattern was formed. In 3 cases follow-up biopsies revealed a time interval of 3 to 5 years for the change-over from stage II–III to stage IV. When cirrhosis was present diffuse cholestasis appeared in all cases.

Chronic-Aggressive Hepatitis – Primary Biliary Cirrhosis Mixed-Types

Patients with chronic inflammatory liver disease, clinical and biochemical evidence of cholestasis, and mitochondrial antibodies to the CAH-PBC mixed-type antigen revealed heterogenous liver changes. Twelve out of 13 cases showed highly active chronic inflammation of the liver frequently accompanied by cirrhosis. The histopathology was characterized by erosions of the periportal parenchyma and large inflammatory bridges dissecting the parenchyma. Another conspicious finding was the coexistence of an intense proliferation of so called atypical ductular structures and of still intact interlobular bile ducts. In some other cases normal bile ducts were virutally absent or were damaged as in PBC I. The latter liver changes which had distinct features of CAH or AC on the one hand and PBC on the other hand, were respectively called CAH-PBC mixed-types or AC-PBC mixed-types. One exception in this series was a chronic portal hepatitis resembling chronic persistent hepatitis (De Groote et al., 1968). Cholestasis, one of the diagnostic pointers of PBC IV, was not always found in the cirrhotic stage of the mixed types.

While cases with the histological features of PBC presented no problems in classifying their liver morphology, it was difficult to find a diagnostic term for the 12 cases with highly active chronic inflammation of the liver and combined features of CAH, AC and PBC. These difficulties are also reflected in the literature. The nomenclature used for cases which appear to have similar changes to those observed here, includes CAH with elevated alkaline phosphatase levels, lupoid hepatitis with cholestasis, active postnecrotic cirrhosis, and cryptogenic cirrhosis (Mackay and Wood, 1962; Datta et al., 1963; MacLachlan et al., 1965; Mackay, 1968; Klatskin and Kantor, 1972; Lüders et al., 1974). Common features are: marked periportal erosion, bridging fibrosis with early transition to cirrhosis, and a distinct proliferation of atypical bile ducts. From

all of these studies it appeares that predominantly middle-aged women are subceptile to this disease. Datta et al. (1963) described a type of postnecrotic cirrhosis in 7 women and 4 men which was characterized by active inflammation, cholestasis, and marked bile ductular proliferation. Moreover, some of these cases showed features of cholangitis and the absence of interlobular bile ducts. An association of destructive lesions of the interlobular bile ducts with criteria of CAH, as observed in some of our cases, were also observed by others (MacLachlan et al., 1965; Schmid, 1966; Sherlock, 1970; Ruckstuhl et al., 1971; Christoffersen et al., 1972; Klatskin and Kantor, 1972; Lüders et al., 1974). Klatskin and Kantor (1972) in a series of 19 patients who had mitochondrial antibodies and had shown PBC lesions in previous biopsies, found all cases with a histological pattern of postnecrotic cirrhosis late in the course of the disease. The same authors reported on the histological characteristics of 8 other patients with mitochondrial antibodies who, in addition to CAH, showed some changes similar to those in PBC. Lüders et al. (1974) observed a chronic periportal hepatitis with marked proliferation of bile ducts and transition to cirrhosis in 9 patients with raised serum alkaline phosphatase and IgM. All were hepatitis B negative and 7 of them had mitochondrial antibodies. Probably some of the cases with so-called chronic necrotizing hepatitis may also belong to this group (Selmair et al., 1970; Wildhirt, 1974). Whether the liver changes, which according to the cited reports and our study might be indicative of the CAH-PBC mixed-type, are indeed restricted to cholestatic liver inflammation of presumed autoimmune nature or also occur to the same extent in non-viral, non-cholestatic CAH or CAH due to viral infection, has to be investigated. As far as we know, there is currently no report or review on the histopathology of CAH which is concerned with this question.

As is evident from our study, the liver histopathology of patients whose serum reacts with the CAH-PBC mixed-type antigen is heterogenous, combining the criteria of CAH-AC and PBC to a varying degree. However, it is still not possible to define the morphologically diagnostic pattern of the CAH-PBC mixed-type. The overlapping of criteria for CAH and PBC is consistent with the view that the destructive process leading to cirrhosis in those cases is directed against both the liver parenchyma and the bile duct system (Doniach and Walker, 1969). Thus the variable characteristics of the CAH-PBC mixed-type morphologically could be explained by the different effects of the destructive process on the two main cell systems of the liver. There are thus three possible effects: (1) if destruction is mainly restricted to the hepatocytes, then highly active CAH and AC with augmented ductular proliferation develops, (2) if destruction is mainly restricted to the bile duct cells, then liver changes predominantly resemble PBC, and finally (3) if hepatocytes and bile duct cells are destroyed to the same degree then true CAH-PBC or AC-PBC mixed-types originate.

Chronic Aggressive Hepatitis without Cholestasis

Patients with chronic inflammatory liver disease, antinuclear and/or anti-smooth muscle antibodies, but without chronic cholestasis or mitochondrial antibodies,

showed the well-known criteria of CAH or AC (Degroote et al., 1968; Boyer, 1976). In CAH ductular proliferation was rarely observed but, when occurring, was generally restricted to single portal tracts. In AC the extent of ductular proliferation also seemed to be much lower than in CAH-PBC mixed-types. With regard to structure and localization of the proliferates we lack sufficient data to differentiate between CAH-AC on the one hand and CAH-PBC mixed-types on the other.

Conclusion

Our research indicates that patients with mitochondrial antibodies to pure PBC antigen have, without exception, liver changes characteristic of PBC. By contrast, patients with mitochondrial antibodies against the CAH-PBC mixed-type antigen showed liver alterations of heterogenous nature. Many cases displayed CAH and/or AC with augmented ductular proliferation. Some of these cases had additional criteria of PBC and were, therefore, considered CAH-PBC of AC-PBC mixed-types. An important clue to the histological differentiation of the latter cases from true PBC was the finding that all stages of PBC overlapped in one biopsy and that these changes were associated with a heavy inflammatory process, even in the cirrhotic stage. The histological separation of CAH-PBC mixed-types from CAH forms without cholestasis and mitochondrial antibodies was primarily based on the occurrence of greatly augmented ductular proliferation in the absence of normal bile ducts. However, it has to be emphasized that the histology of one of the cases whose serum was positive with the CAH-PBC mixed-type antigen was that of PBC and another that of chronic persistent hepatitis. Because of our inability to define distinct diagnostic criteria for the liver changes in patients bearing antibodies against the CAH-PBC mixed-type antigen, we suggest that this group represents a partial overlapping of CAH and PBC. However, the question whether CAH-PBC is an "overlapping syndrome" or a true entity can only be resolved by further histological, serological and clinical studies with a larger number of cases.

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Received September 30, 1976