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Pathology of septum formation in primary biliary cirrhosis: a histological study in the non-cirrhotic stage

Yasuni Nakanuma

Second Department of Pathology, Kanazawa University School of Medicine, Kanazawa 920, Japan

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Summary. Bridging or incomplete septum formation, an important step leading to cirrhosis in various chronic progressive liver diseases, was examined in 231 liver biopsy specimens of primary biliary cirrhosis (non-cirrhotic stage). Incomplete septa from the enlarged portal tracts and portal to portal bridges were frequent and appeared first, while portal to central ones appeared subsequently and became frequent in the liver specimens with changes resembling cirrhosis. These septa were divided into four types histologically: ductular, lymphocytic, loose connective tissue and fibrous type. More than one type was usually found in the same specimen. The pathology of the first three types was similar to and frequently continuous with that of neighbouring periportal regions, suggesting that most of these septa were formed by the extension of periportal destructive processes. The fibrous type might be an advanced form of the other three types. Incomplete septa seemed to pinch off part of the hepatic parenchyma in a hepatic lobule; this was followed by an unusual enlargement of the portal tracts and an approximation of portal tracts and central veins. There were perivenular hepatocellular necroses on occasion. Progression of periportal hepatocellular damage may lead to septum formation and finally progress to cirrhosis, in primary biliary cirrhosis. The significance of perivenular necroses remains speculative.

Key words: Primary biliary cirrhosis – Periportal necrosis – Bridge formation – Cirrhosis

Introduction

Primary biliary cirrhosis (PBC) is characterized by non-suppurative destruction of the intrahepatic small bile ducts. In addition, there are many pathological changes in the liver in PBC (Ludwig et al. 1978; Scheuer 1980; Portmann et al. 1985; Portmann and MacSween 1987; Nakanuma et al. 1990). Among the latter, cirrhotic transformation or advanced hepatic fibrosis is an ominous and terminal pathological feature of PBC, shortly followed by oesophageal bleeding varices or heaptic failure (Sherlock 1989). Popper (1981) suggested that there may be at least two pathological mechanisms of hepatocellular damage in PBC (Schaffner and Popper 1982): cholestatic liver cell damage (probably due to bile duct loss and interference of bile flow) and chronic active hepatitis (CAH)-like liver cell damage (probably reflecting autoimmune hepatocellular damage). These two processes might have been operative in PBC livers in variable combinations (Popper 1981; Portmann et al. 1985; Nakanuma et al. 1990). However, the exact morphological processes leading to cirrhotic transformation in PBC remain unclear.

In other chronic progressive liver diseases such as CAH, septum formation associated with hepatocellular loss and regeneration is an important step in their pathogenesis. It is followed by lobular distortion, intrahepatic micro-circulatory disturbance and finally cirrhotic transformation (Scheuer 1980, 1988; Cooksley et al. 1986). As to PBC, there have been no systematic or detailed morphological studies on septum formation and progression to cirrhosis to the best of our knowledge. In this study, detailed morphology of the septum formation in non-cirrhotic stages of PBC was examined using a considerable number of liver biopsy specimens.

Materials and methods

The liver specimens of PBC were classified histologically into two stages: non-cirrhotic and cirrhotic. The former was histologically subdivided into six groups (A–F). Briefly, group A was characterized by sharply and well-preserved limiting plates. The lobular architecture was well-preserved, and lymphoid cell infiltration in the portal tracts was variably found, and differed from one portal tract to another. Non-suppurative destructive cholangitis was also seen in some portal tracts. Group B showed focal disruption of the limiting plates in some portal tracts. Otherwise, group B shared the histological features of group A. In group C, more than half of the portal tracts in liver specimens showed periportal changes

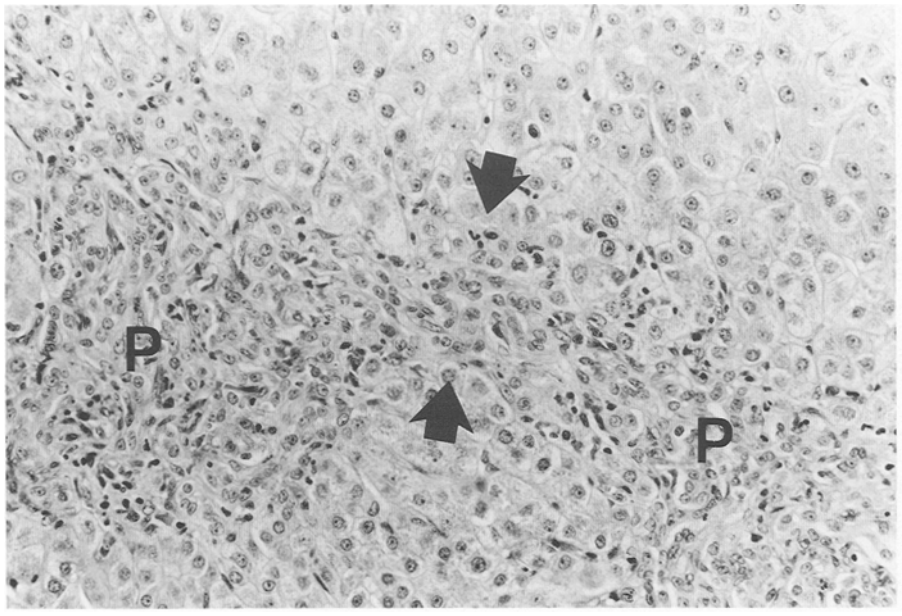

Fig. 1. Portal-portal bridge of ductular type shown by *arrows*. There is also atypical ductular proliferation in the adjacent portal tracts. *P*, Portal tract. Hematoxylin and eosin (H & E) stain, ×625

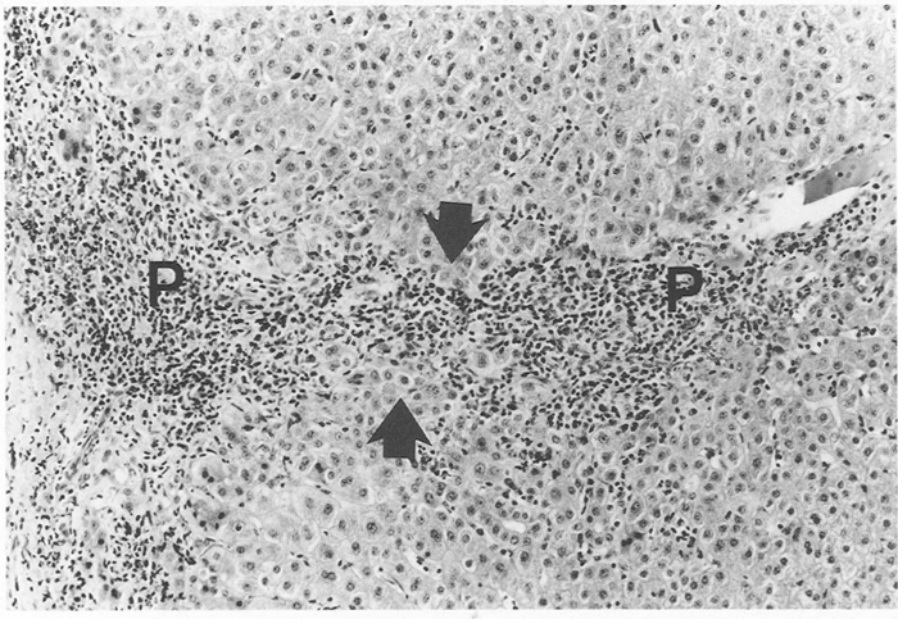

Fig. 2. Portal-portal bridge of lymphocytic type (*arrows*). There is also lymphocytic piecemeal necrosis in the adjacent periportal areas. *P*, Portal tract. H & E, ×360

(destruction of the limiting plates) associated with several pathological changes such as ductular proliferation (vide infra). Septum or bridge formation was totally absent in this group. Group D showed the formation of a few septa focally with variable periportal changes. Lobular architecture was well-preserved in groups C and D. Group E showed a variable number of bridges formed in the liver specimen, and also variable periportal and periseptal changes. There was variable disturbance of lobular architecture. Group F showed dense scar-like fibrous septa additionally, which were absent in group E. Group F had changes approaching cirrhosis. These six groups appeared to progress step-wise from A to F as proposed in classical staging systems (Ludwig et al. 1978; Scheuer 1980), though there is no convincing data on progression. Cirrhotic stage was characterized by regenerative nodules.

A total of 231 liver biopsy specimens of non-cirrhotic stages from 216 PBC patients (range of ages: 28–76 years; female to male ratio, 203:13) were obtained from many institutions in Japan, including the Kanazawa University Hospital (1966–1990), for analysis of PBC. Eighty-five liver biopsies were performed percutaneously and 146 at laparotomy. These liver specimens were divided into six groups as follows: group A, 27 specimens; group B, 43 specimens; group C, 34 specimens; group D, 57 specimens; group E, 46 specimens; and group F, 24 specimens. A diagnosis of PBC was made by a combination of clinical, laboratory and histological findings (Sherlock 1989). Liver biopsy specimens from the patients under the long-term administration of ursodeoxycholic acid were not included in the present material because this form of therapy is known to reduce or modify the necro-inflammatory changes in PBC (Poupon et al. 1987; Matsuzaki et al. 1990). All livers were fixed in 10% neutral formalin and embedded in paraffin. Several 5-μm sections from these specimens were stained with haematoxylin and eosin, Azan-Mallory, Gomori's reticulin and orcein stains (Nakanuma et al. 1979). All specimens were examined without knowledge of the clinical and laboratory background.

Septum formation in which well-formed interlobular bile ducts or hepatic arterial branches were absent was subdivided into bridg-

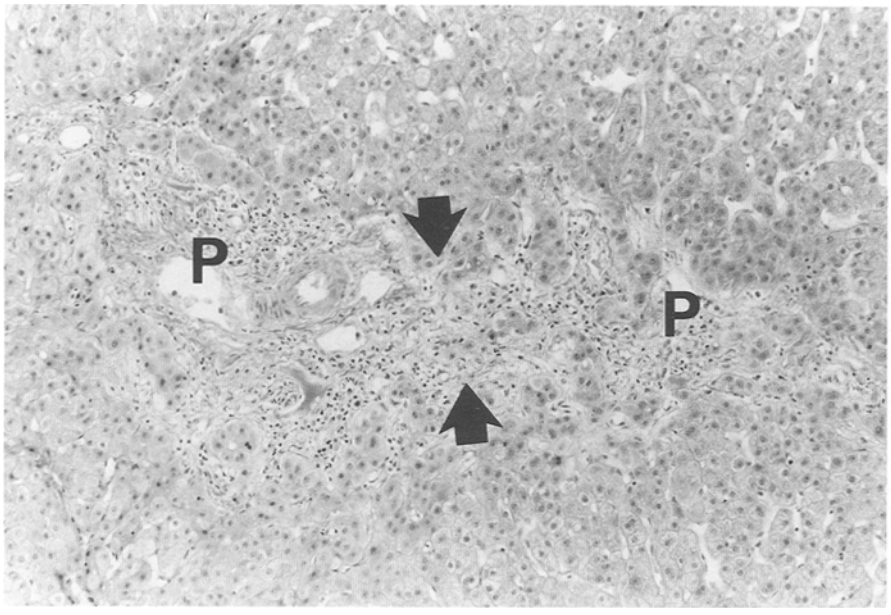

Fig. 3. Portal-portal bridge of loose connective tissue type (*arrows*). There is also a mild degree of lymphocytic reaction in this bridge. Loose connective tissue extends into periportal regions. *P*, Portal tract. H & E, ×360

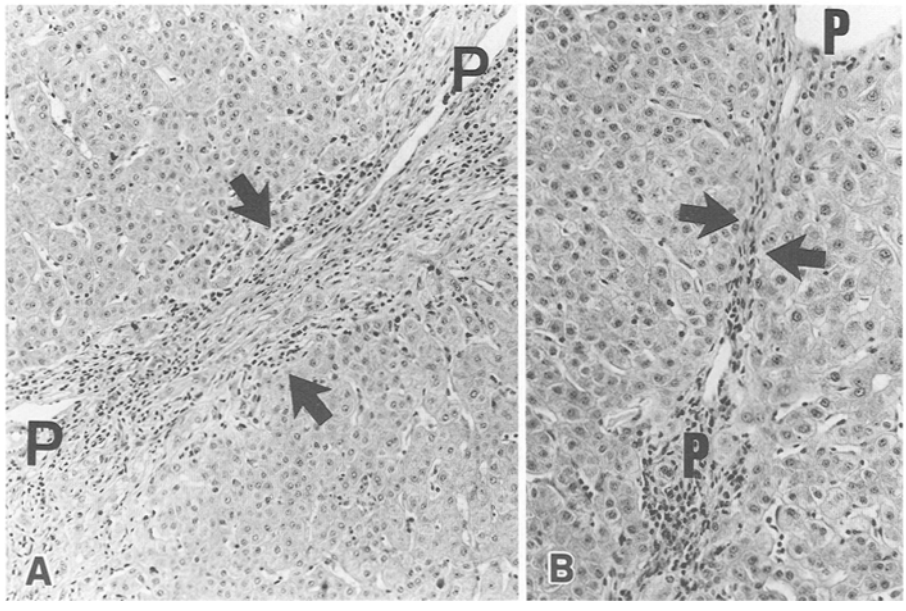

Fig. 4. A Fibrous bridge of broad type shown by *arrows*. There is also some ductular and lymphocytic reaction. *P*, Portal tract. H & E, ×280. **B** A bridge of fibrous type showing thin or collagenous band (*arrows*). This type may represent an old or quiescent form of the other three types of bridges. *P*, Portal tract. H & E, ×360

ing and incomplete. Bridging septa were recorded as being either portal-central (P-C) where bridges existed between portal tracts and central veins, portal-portal (P-P) where bridges were present between adjacent portal tracts, or central-central (C-C) where linkage was between adjacent central veins. An incomplete septum was a bud-like extension of the enlarged portal tracts into the hepatic lobule which did not reach the adjacent portal tracts or central veins. The length and width of incomplete septa were variable. Proliferated bile ductules were classified into two types, typical and atypical, by their shape and location (Scheuer 1988). Typical ductules were well-formed with definite lumen and were usually cut in cross-section. Atypical ductules were characterized by an elongated or anastomosing configuration with poorly defined lumen and small cytoplasmic volume and they were usually crowded and adjacent to the hepatic parenchyma. They were usually cut longitudinally.

Periportal changes associated with disruption of the limiting plates were classified into four types histologically according to our previous study (Nakanuma and Ohta 1980): ductular, lymphocytic, loose connective tissue and fibrous types. In ductular type, shaggy periportal parenchyma was intermingled with proliferated atypical bile ductules or ductular transforming hepatocytes. The lymphocytic type was characterized by destruction of the limiting plates and lymphoplasmacytic infiltration. Loose connective tissue type was defined as destruction of the limiting plates in which hepatocytic cords were separated by loose connective tissue, resembling oedema. The fibrotic type was characterized by shaggy-limiting plates and invasion of dense slender fibrous tissue between the hepatocellular cords.

Some areas of hepatic parenchyma showing multicellular thickness or vague nodules without a fibrous rim (Nakanuma and Ohta 1987) were regarded as active regeneration. In addition, when cellular and nuclear size or cytoplasmic staining of hepatocytes were different from one parenchymal area to the adjacent one as reported in CAH (Peters 1978), such change was also regarded as a finding of active regeneration.

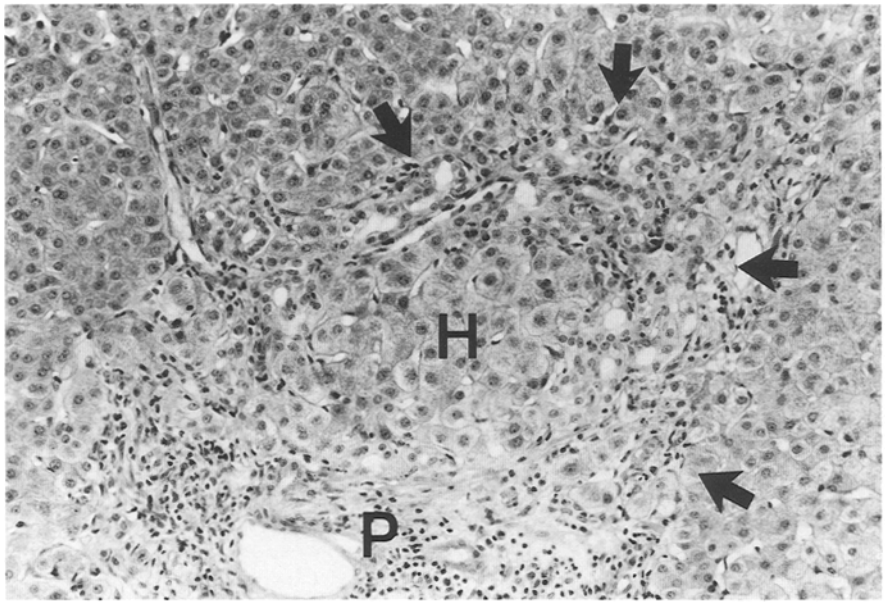

Fig. 5. Pinchin-off phenomenon of incomplete septa. A part of the hepatic parenchyma (*H*) is partitioned by septa of atypical ductules (*arrows*). H & E, ×600

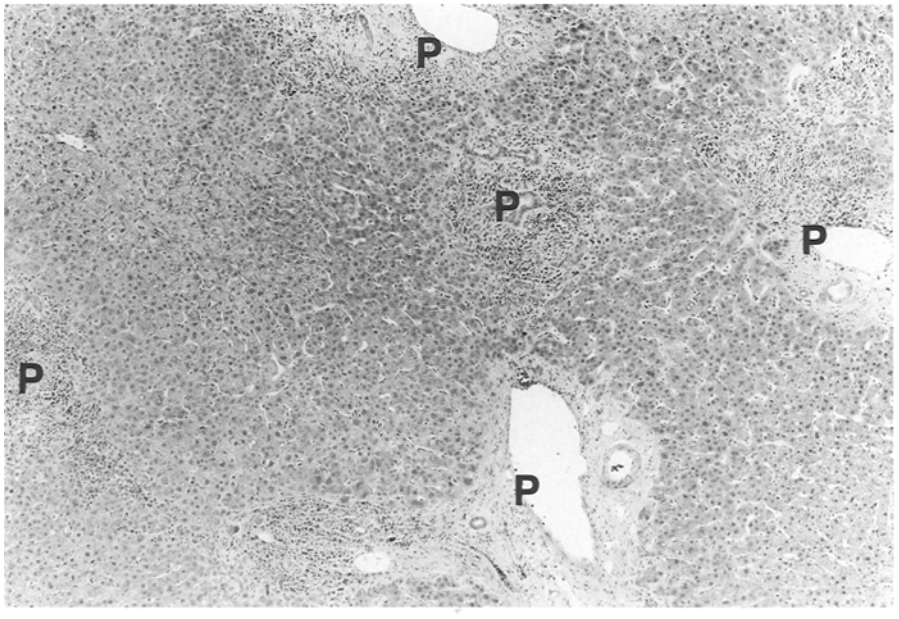

Fig. 6. Unusual approximation of adjacent portal tracts (*P*) in the middle and right part of this figure. H & E, ×200

Results

P-P or P-C bridges were histologically classified into four types; ductular, lymphocytic, loose connective tissue and fibrous types (Figs. 1–4). Some bridges were, however, composed of more than one type, so that distinction from each other was arbitrary on occasion. More than one type was usually found in a variable combination in the same specimen. The ductular type was characterized by the presence of atypical bile ductules in the bridges (Fig. 1) and was associated with a variable mixture of inflammatory cells. Some adjoining hepatocytes were undergoing ductular transformation, and some of these transformed ductules showed xanthomatous changes. In lymphocytic type, a considerable number of lymphocytes admixed with plasma cells were found within the bridge and also between hepatocytes facing the bridge (Fig. 2). Loose connective tissue type suggesting oedema was associated with no or few inflammatory cells (Fig. 3). These three types appeared as a rather thin band and the small amount of fibrous tissue associated with either of the three types, when present, was not dense. On contrast, the fibrous type was characterized as a rather broad and dense fibrous band with variable ductules and inflammatory cells (Fig. 4A) or a thin dense collagenous bridge with few ductules and lymphocytes (Fig. 4B). The characteristics of these pathological changes in the bridging and incomplete septa were similar to those of adjacent periportal lesions (Figs. 1–3).

Incomplete septa also showed some of the features of the above-mentioned four lesions. Two incomplete septa radiating from an enlarged portal tract occasional-

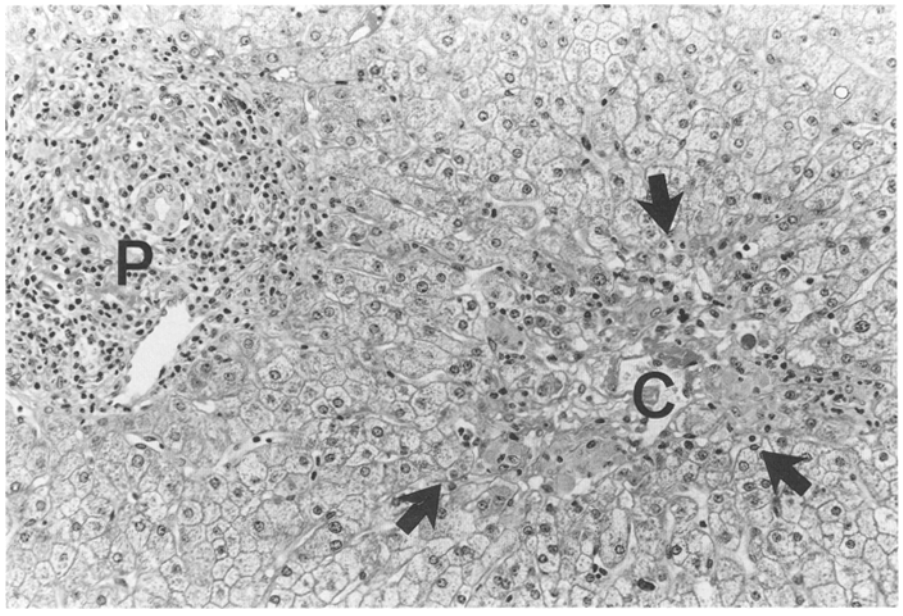

Fig. 7. There is zonal loss of hepatocytes in the perivenular region (perivenular necrosis, *arrows*) with a few acidophilic bodies and epithelioid cells. *C*, Central vein; *P*, Portal tract. H & E, ×360

Table 1. Location of bridging septa (positive/examined specimens)

Group	P-P	P-C	C-C
A	0/27	0/27	0/27
B	0/43	0/43	0/43
C	0/34	0/34	0/34
D	55/57	14/57	5/57
E	46/46	26/46	0/46
F	24/24	20/24	0/24

P-P, Portal to portal; P-C, portal to central; C-C, central to central bridge; groups A–F, see text

Table 2. Histological type of portal to portal and portal to central bridge (positive/examined specimens)

Groups	Ductular	Lymphocytic	Loose connective tissue	Fibrous
A	/	/	/	/
B	/	/	/	/
C	/	/	/	/
D	21/57	31/57	17/57	23/57
E	20/46	33/46	12/46	25/46
F	14/24	11/24	14/24	15/24

/, no lesion; groups A–F, see text

ly seemed to connect with each other within a hepatic lobule and to pinch off a part of the lobule (Fig. 5). Such pinched off small-sized areas of hepatic parenchyma seemed to be incorporated into the enlarged portal tract as a trapped hepatocellular cluster. There were, on occasion, unusual approximations of neighbouring portal tracts or central veins (Fig. 6).

In 6 cases where infection of hepatitis A or B virus was excluded serologically, there were focal necroses accentuated in perivenular regions (Fig. 7) appearing as perivenular zonal necrosis seen in acute hepatitis. Anti-hepatitis C virus antibody was negative in the 4 cases examined. These necroses were usually associated with scattered acidophilic bodies, pigmented macrophages, and occasional epithelioid cells. A variable perivenular fibrosis was also found in all of these cases.

By definition, there were no cases showing septa formation in groups A, B and C (Tables 1, 2). The limiting plates were, however, variably disrupted and showed the periportal changes in groups B and C (see above). By definition, there were incomplete septa of variable length from enlarged portal tract and a few bridges (mainly, P-P type) in group D. P-C and C-C bridges were also seen infrequently (24.6% and 8.8%, respectively). In groups E and F, bridging and incomplete septa became increased and wider, and P-P bridges were found constantly. P-C bridges were more frequent in group F than in group E. The distribution and degree of these bridging and incomplete septa were uneven in group D and E, though they became widespread in group F.

Four histological types of bridging or incomplete septa were found in groups D, E and F. There were no characteristic distribution of these four types with respect to group D, E or F.

Sequential observations of liver biopsies (more than three times) were made in 5 cases (Table 3). In cases 1, 2 and 3, P-P bridges were seen in the first biopsy, and P-C bridges developed sequentially. In case 4, however, P-C bridges in the first and second biopsies disappeared in the third biopsy. P-P bridges themselves in the first and second biopsies became unclear in the third biopsy. While the histological types of bridge seemed to persist in each case, there were considerable variations.

Table 3. Sequential observations of location and histological type of portal to portal and portal to central bridge (positive/examined specimens)

Case 1	(Group)	(Location)	Ductular	Lymphocytic	Loose connective tissue	Fibrous
1st	(D)	(P-P)	++	+	−	+
2nd	(D)	(P-P, P-C)	−	+	−	+
3rd	(F)	(P-P, P-C)	+	−	+	−
Case 2	(Group)	(Location)	Ductular	Lymphocytic	Loose connective tissue	Fibrous
1st	(D)	(P-P)	−	+	+	−
2nd	(E)	(P-P, P-C)	−	++	−	−
3rd	(E)	(P-P, P-C)	−	+++	−	−
Case 3	(Group)	(Location)	Ductular	Lymphocytic	Loose connective tissue	Fibrous
1st	(D)	(P-P)	++	−	−	−
2nd	(E)	(P-P, P-C)	−	+	+	++
3rd	(F)	(P-P, P-C)	+	−	−	++
Case 4	(Group)	(Location)	Ductular	Lymphocytic	Loose connective tissue	Fibrous
1st	(E)	(P-P, P-P)	−	++	−	−
2nd	(E)	(P-P, P-C)	−	++	−	−
3rd	(D)	(P-P)	−	−	−	+
Case 5	(Group)	(Location)	Ductular	Lymphocytic	Loose connective tissue	Fibrous
1st	(D)	(P-P)	+	+	−	−
2nd	(D)	(P-P)	−	−	−	+
3rd	(A)	/	/	/	/	/

1st, 2nd, and 3rd, 1st liver biopsy, 2nd liver biopsy, and 3rd liver biopsy, respectively; −, negative or minimum; +, mild; ++, moderate; +++, marked degree; /, no lesion: A-F, see text

Discussion

The pathogenesis and significance of septum formation may differ from disease to disease in various chronic progressive liver diseases (Scheuer 1980, 1988; Cooksley 1986). For example, P-P linkage has a different pathogenesis from P-C bridging in CAH, and the latter reflects a rapid progression to cirrhosis (Cooksley 1986). It was shown in the present study that in PBC, P-P bridge was common relative to P-C bridges and appeared to develop first. The latter became evident and frequent in liver specimens approaching cirrhosis. Incomplete septa from the enlarged portal tracts may be a forerunner of P-P and P-C bridges. Scheuer (1988) reported that linking of portal tracts to each other was common in conditions where portal tracts were widened in chronic biliary diseases, supporting our observation.

It was also found in this study that P-P or P-C bridges and incomplete septa were composed of four types of histological changes (ductular, lymphocytic, loose connective tissue and fibrous type). It was of interest that these four changes, especially the first three, were basically similar to morphologies of destructive lesions at the limiting plates (Nakanuma and Ohta 1980; Portmann et al. 1985). These periportal changes were continuous here and there with those in the septa. Thus, it seems likely that periportal damage with hepatocellular injury extends into the hepatic lobule and leads to the development of bridging or incomplete septa. This process may progress to cirrhosis as these bridges become wider and fibrotic.

Pathogenetically, the ductular type may be related to chronic long-standing cholestasis (Popper 1981; Nakanuma and Ohta 1986; Scheuer 1988). The lymphocytic type is probably related to autoimmune hepatocellular necrosis. The fact that subsets of infiltrating lymphocytes in these portions are similar to those of CAH (Van den Oord et al. 1984; Nakanuma et al. 1990) also supports this suggestion. The pathogenesis of the loose connective tissue type remains speculative, but may be related to chronic cholestasis (Popper 1981). These three types of bridges seem to be associated with hepatocellular damage or necrosis and correspond to bridging necrosis in PBC, originally reported by the Mayo group (Baggenstoss et al. 1972; Ludwig et al. 1978; Dickson et al. 1979). The fibrous type may be an advanced or old form of the three types of bridge.

In PBC livers, on unusual approximation of adjoining portal tracts or central veins without bridges were occasionally found. In addition, adjacent enlarged portal

tracts were frequently connected, where well-formed vascular or biliary elements were found. Enlarged portal tracts cut longitudinally were frequently found in wedge biopsy specimens. These features may represent on-going periportal hepatocellular loss and progression of the pinching off phenomenon with loss of trapped hepatocytes in the enlarged portal tracts.

The present study also showed that bridging or incomplete septa occur unevenly in the liver in PBC, especially in groups D and E. It was also of interest that some portal tracts were severely damaged whereas others were almost normal in the same specimen in groups C and D. There were actually variably sized nodules of hepatic parenchyma found in the non-cirrhotic stage, suggesting that extenstive damage similar to septum formation affects a considerable area of hepatic lobules. Baggenstoss et al. (1972) reported that 2 of 7 cases of PBC represented subacute hepatitis with bridging. Such cases may correspond to PBC livers with extensive bridges. These data suggest that periportal lesions followed by bridge development or incomplete septum formation and even focal collapse do not occur synchronously within the hepatic parenchyma, although this irregular parenchymal damage seems to become widespread and homogeneous with histological progression.

It was of interest that perivenular zonal necrosis was evident in a few cases in which C-C and to a lesser extent P-C bridging was also found. These changes are apparently similar to those found in acute viral or drug-induced hepatitis (Peters 1978) though there was a variable perivenular fibrosis and occasional epithelioid cell reaction in our cases. Simultaneous presence of perivenular zonal necrosis and a variable perivenular fibrosis in the same specimen suggest that such hepatocellular necrosis occurs repeatedly. This has not been documented in the English and Japanese literature. Viral markers were negative in the cases examined, raising the possibility that perivenular necrosis is an inherent pathological change in PBC.

On-going necro-inflammatory changes in periortal regions in PBC may be followed by incomplete and bridging septum formation, an important step to the development of cirrhosis. The pathology of these septa may be divided into four categories, which may show different responses to various therapies such as ursodeoxycholic acid and immunosuppressive drugs (Poupon et al. 1987; Matsuzaki et al. 1990).

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