Virchows Arch (1998) 433:471–479 © Springer-Verlag 1998

ORIGINAL ARTICLE

Masashi Yamashiro · Wataru Kouda · Naoko Kono
Koichi Tsuneyama · Omasu Matsui
Yasuni Nakanuma

Distribution of intrahepatic mast cells in various hepatobiliary disorders

An immunohistochemical study

Received: 2 January 1998 / Accepted: 29 May 1998

Abstract There is evidence that mast cells are involved in a number of pathophysiological processes. The significance of mast cells in hepatic fibrosis was examined in 28 patients with histologically normal livers, 34 with acute liver diseases, 51 with chronic liver diseases, and 59 with cholestatic biliary diseases, using immunostaining of the mast cell-specific proteinase, tryptase. Mast cells that were positive for tryptase and for chymase were significantly increased in frequency in fibrotic portal tracts and fibrous septa, particularly in cholestatic/biliary diseases. Mast cells were also increased in frequency around the fibrotic septal and intrahepatic large bile ducts and peribiliary glands of biliary diseases. However, they were less common or even rare in the sclerotic bile ducts and in scarred portal or septal fibrosis. More than half of these more numerous mast cells were positive for histamine, and some were also positive for basic fibroblast growth factor. These two substances were detectable by immunoelectron microscopic in the cytoplasmic granules of mast cells. In contrast, mast cell numbers were not significantly increased in acute viral or drug-induced hepatitis, or in zones 2 and 3 of the hepatic acinus with respect to pericellular and perivenular fibrosis in chronic liver diseases. These findings suggest that mast cells increase in number in cholestatic/biliary diseases, and to a lesser degree in chronic liver diseases, and are involved in the active fibrous enlargement of portal tract and fibrous septa formation and also in the fibrosis of the intrahepatic bile ducts as they display fibrosis-promoting factors such as tryptase, fibroblast growth factor and histamine.

M. Yamashiro · W. Kouda · N. Kono · K. Tsuneyama
Y. Nakanuma (✉)
Second Department of Pathology,
Kanazawa University School of Medicine, Kanazawa 920, Japan
Tel.: +81-76-265-2197, Fax: +81-76-234-4229

O. Matsui
Department of Radiology,
Kanazawa University School of Medicine, Kanazawa 920, Japan

Key words Mast cells · Hepatic fibrosis · Immunohistochemistry · Tryptase · Basic fibroblast growth factor

Introduction

Active investigations into the role of mast cells (MCs) in various inflammatory, fibrotic and proliferative disorders and angiogenesis have been undertaken [9, 20, 21, 29, 37] and have shown that human MCs promote fibroblast growth and collagen synthesis and affect the organization of connective tissue elements in several organs [9, 16, 17, 22, 29, 31] by producing and secreting bioactive mediators contributing to fibrosis. These mediators include tryptase [33], tumour necrosis factor-α [3,14], IL-1 [4] and transforming growth factor-β [15]. More importantly, recent studies [19, 29] suggest that MCs are a major source of basic fibroblast growth factor (bFGF).

MCs have been identified in normal human livers [8] and in some granulomatous liver diseases [5]. A recent study (W. Kouda et al., submitted) disclosed that in normal livers, MCs are few in number in portal tracts and hepatic parenchyma, but densely and regularly distributed around the intrahepatic biliary tree. We suggest that the latter are resident MCs in a normal human liver. During hepatic fibrogenesis [6, 12, 25], stellate cells (fat-storing cells) and bile ductular cells are known to secrete fibrosis-related mediators and are regarded as the primary source of collagen and other extracellular matrix components. Recently, Farrel et al. [8] showed quantitatively that intrahepatic MCs are also involved in portal fibrosis in alcoholic liver diseases and primary biliary cirrhosis (PBC). Armburst et al. [1] stressed that MCs may inhibit extracellular matrix degradation by displaying protease inhibitors. However, the exact pathologic roles of MCs and their bioactive substances, particularly fibrogenetic mediators, have not been fully examined.

We first examined the distribution of intrahepatic MCs in various hepatobiliary diseases, and secondly attempted to analyse their pathobiological role in fibrosis

using primary antibodies against tryptase, bFGF, and histamine, which are known as potent fibroblast growth factors [19, 29, 33].

Materials and methods

Liver tissue specimens (needle or wedge biopsied, surgically resected, and autopsied liver specimens) registered in our file of liver diseases were used. In the autopsy and surgically resected specimens, the hepatic hilar regions were also processed for tissue preparation. All specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Their number and main clinical features are shown in Table 1.

Acute drug-induced hepatitis was histologically similar to acute viral hepatitis. The aetiology of acute viral hepatitis was HAV related in 15 cases and HCV related in the remaining 4 cases; the two diseases were grouped together as acute liver disease. Chronic viral hepatitis (nonchirrhotic) was HCV related in 28 cases, while the remainder were HBV related. These were staged according to the degree of fibrosis [7]: F_0, no portal fibrosis; F_1, fibrous portal expansion; F_2, bridging fibrosis, F_3, bridging fibrosis with architectural distortion. Liver cirrhosis was HBV related in 2 cases and HCV related in 8. Eight cases of alcoholic liver fibrosis showed moderate portal and perivenular fibrosis, with a few cases of portal-to-central and central-to-central bridging fibrosis and moderate fatty change; alcoholic hepatitis or cirrhosis was not included. Chronic viral hepatitis, liver cirrhosis, and alcoholic fibrosis were grouped as chronic liver disease. In 8 cases of hepatolithiasis, all hepatoliths were localized to the intrahepatic biliary tree and were of calcium bilirubinate stones. PBC was histologically staged according to Scheuer [36]: I, II, III, and IV. In primary sclerosing cholangitis (PSC) moderate portal fibrosis was seen with portal-to-portal bridging fibrosis, and also cholestasis in 14 cases, the remaining 2 cases being cirrhotic. The duration of jaundice in 8 cases of extrahepatic biliary obstruction (EBO) was under 3 months (5 cases due to biliary tract carcinoma, 2 cases due to pancreatic carcinoma and the remainder due to choledocholithiasis). PBC, PSC, heaptolithiasis and EBO were grouped together as cholestatic/biliary disease.

Twenty-four liver specimens were fixed in AMeX (acetone, methyl benzoate, and xylene; Table 1) [35]. These were included with the formalin-fixed specimens; liver tissue specimens were fixed in acetone at –20° C overnight, then cleared in methyl benzoate and xylene and embedded in standard paraffin. This fixation was devised as a new simplified method of immunostaining using both monoclonal and conventional polyclonal antibodies. In addition, tissue and cellular structures were well preserved by this procedure to a standard comparable to that of formalin fixation.

More than 20 4-μm-thick sections were cut from each paraffin block. Some were processed for routine stainings including H&E. The remainder were used for immunohistochemistry with the primary and secondary antibodies shown in Table 2.

Our preliminary study showed that after formalin or AMeX fixation, the antigenicity of tryptase was well preserved. Chymase, bFGF and histamine were detectable more clearly and reproducibly after AMeX fixation. In accordance with the method published by Inoue et al. [19], we adapted monoclonal antibody against bovine bFGF type II to detect human bFGF. MCs were surveyed and quantitated in formalin-fixed sections immunostained for tryptase, a specific and sensitive marker of MCs [19]. The immunohistochemical detection of tryptase, chymase, bFGF and histamine was also done in the AMeX fixed sections for further characterization of MCs.

A standard avidin–biotin complex–peroxidase (ABC/PO) method involving a Vectastain ABC kit (Vector, Burlingame, USA) was used. After abolition of endogenous peroxidase and incubation in nonimmune serum, the sections were incubated at 4° C overnight with primary antibodies. The sections were then treated for 45 min at room temperature with biotinylated secondary antobidies and then in the ABC/PO (Vectastain ABC kit, Vector) for 45 min at room temperature. Peroxidase activity was visualized by the benzidine reaction. Nuclei were counterstained with methyl green.

Double immunostaining of tryptase and bFGF in MCs was done using AMeX-fixed paraffin sections (1 normal, 2 cirrhotc, and 1 hepatolithiatic livers). The sections were incubated with primary antibody to tryptase (diluted 1:200) and then treated with secondary antibodies to mouse IgG (horse). Thereafter, ABC-alkaline phophatase (ABC/AP; Vector) was applied and the sections were visualized with the alkaline phosphatase substrate kit 1 containing fast red (Vector Lab) with one drop of levamisole (1.25 mmol/l, Vector). To abolish and inactivate both the primary and the secondary antibodies applied, the sections were washed in running water and then incubated in hot water (90° C) for 10 min [2].

Table 1 Liver tissue specimens used and their main clinicopathological features (*AMeX* fixation in acetone, methyl benzoate, and xyline (35), *A* autopsy, *S* surgically resected, *W* wedge biopsy, *N* needle biopsy, $F_{0–3}$ staging of chronic hepatitis (F_0 no portal fibrosis, F_1 fibrous portal expansion, F_2 bridging fibrosis, F_3 bridging fibrosis with architectural distortion) according to Desmet [7]; staging of primary biliary cirrhosis is in accordance with Scheuer [36])

Type of liver	No. of cases	Age range (years)	Male:female	Fixation in formalin (and AMeX)
Histologically ''normal livers'' (A 15; S 5; N 8)	28	35–70	15:13	28(10)
Acute viral hepatitis (all N)	19	23–50	12:7	19
Acute drug-induced hepatitis (A 1; N 14)	15	31–70	10:5	15
Chronic viral hepatitis				
Noncirrhotic (W 3; N 30) (F_0 3; F_1 5; F_2 20; F_3 5)	33	28–67	23:10	33
Cirrhotic (N 5; A 5)	10	54–70	7:3	10(4)
Alcoholic liver fibrosis (A 2; N 6)	8	54–70	5:3	8 (2)
Primary biliary cirrhosis				
Stages I+II (W 8; N 12)	20	34–63	18:2	20(1)
Stages III+IV (W 4; N 3)	7	40–66	6:1	7(1)
Primary sclerosing cholangitis (A 2; S 4; W 6; N 4)	16	27–71	9:7	16(1)
Hepatolithiasis (all S)	8	35–58	4:4	8(1)
Extrahepatic biliary obstruction (A 8)	8	53–75	5:3	8(2)

Table 2 Primary and secondary antibodies used and their optimal dilution (*F* formalin fixed, *mono* monoclonal, *poly* polyclonal)

Antibodies against	Source	Animals immunized	Type of antibody	Suitable fixation	Optimal dilution
Primary antibodies					
Mast cell tryptase (AA1)	DAKO, Glostrup, Denmark	Mouse	mono	F, AMeX	1:200
Mast cell chymase	Chemicon, Tamecula, USA	Mouse	mono	AMeX	1:200
Bovine basic fibroblast growth factor, type II	Upstate Biotechnology, Lake Placid, USA	Mouse	mono	AMeX	1:200
Histamine	Chemicon, Tamecula, USA	Rabbit	poly	AMeX	1:200
Biotinylated secondary antibodies					
Rabbit IgG	Vector, Burlingame, USA	Goat			1:200
Mouse IgG	Vector, Burlingame, USA	Horse			1:200
Fluorescein-conjugated secondary antibodies					
Mouse IgG	Chemicon, Tamecula, USA	Horse	poly		1:200

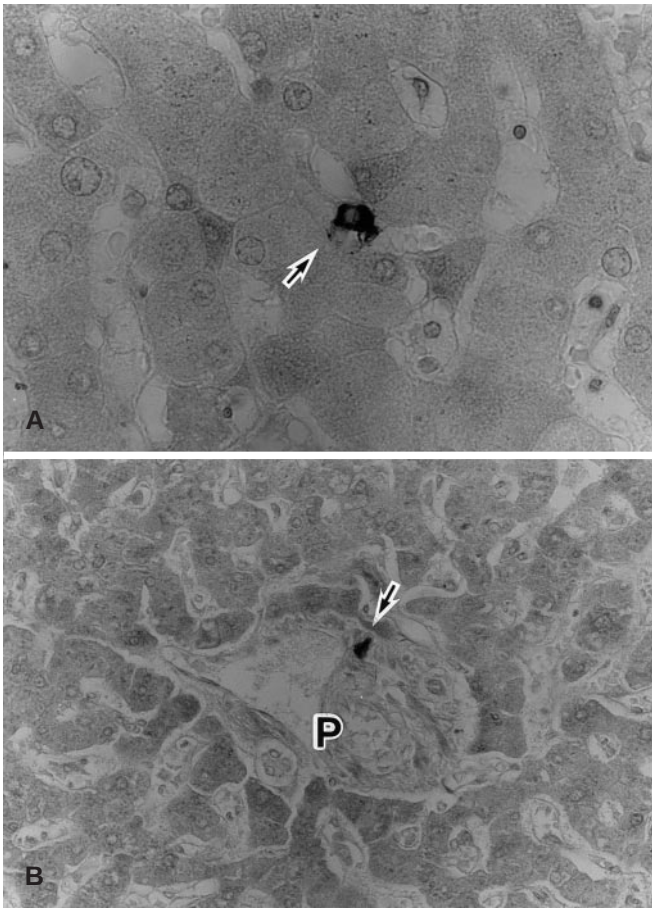


Fig. 1 **A** One tryptase-positive mast cell (*arrow*) is found in the sinusoid of hepatic acinus (zone 2). Normal liver. Immunostaining of tryptase (ABC method) and haematoxylin, ×350 **B** One tryptase-positive mast cell (*arrow*) is found in a portal tract. Normal liver (*P* portal tract). Immunostaining of tryptase (ABC method) and haematoxylin, ×300

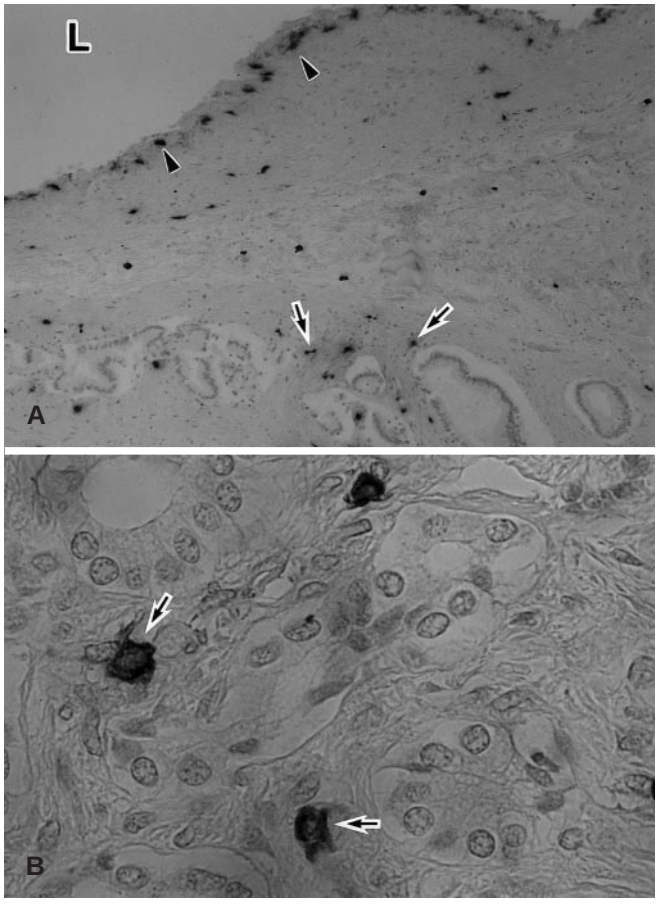
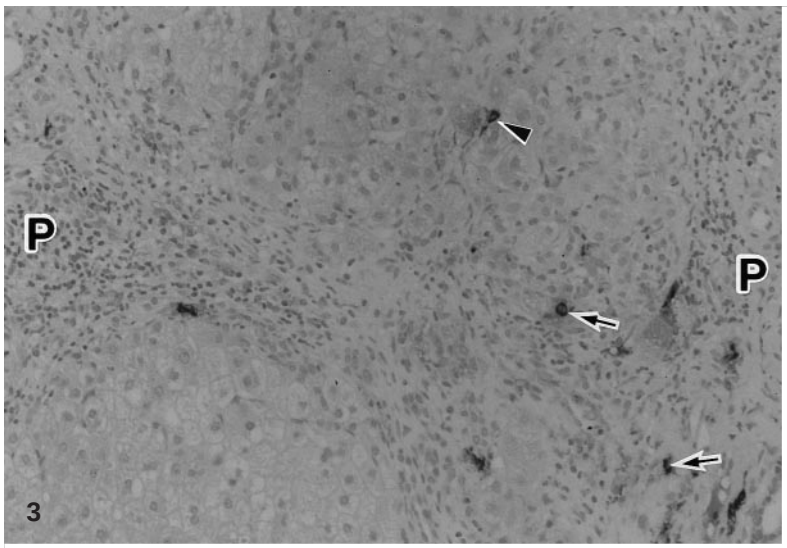


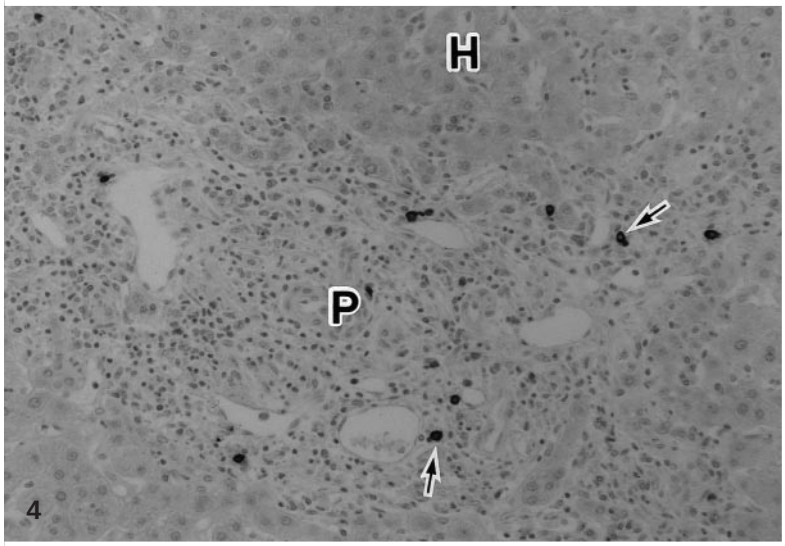
Fig. 2 **A** Tryptase-positive mast cells are found beneath the biliary epithelial layer (*arrowheads*) and also in the peribiliary glands (*arrows*; *L* bile duct lumen). Immunostaining of tryptase (ABC method) and haematoxylin, ×100 **B** Tryptase-positive mast cells (*arrows*) are found within one lobule of peribiliary gland. Immunostaining of tryptase (ABC method) and haematoxylin, ×350

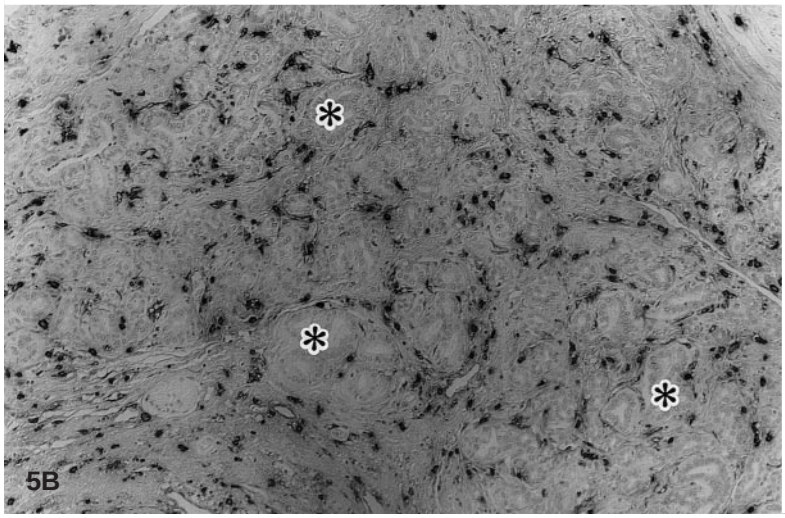
Then, the sections were incubted with the primary antibody to bFGF (diluted 1:200). Next, the sections were incubated with fluorescein-conjugated anti-mouse IgG horse antibody. Tissue sections were examined under confocal laser microscope (LSM410, Carl Zeiss, Göttingen, Germany): tryptase was recognizable as red (argon laser 710 nm for Vecta red) and bFGF as green (argon laser 492 nm for fluorescein).

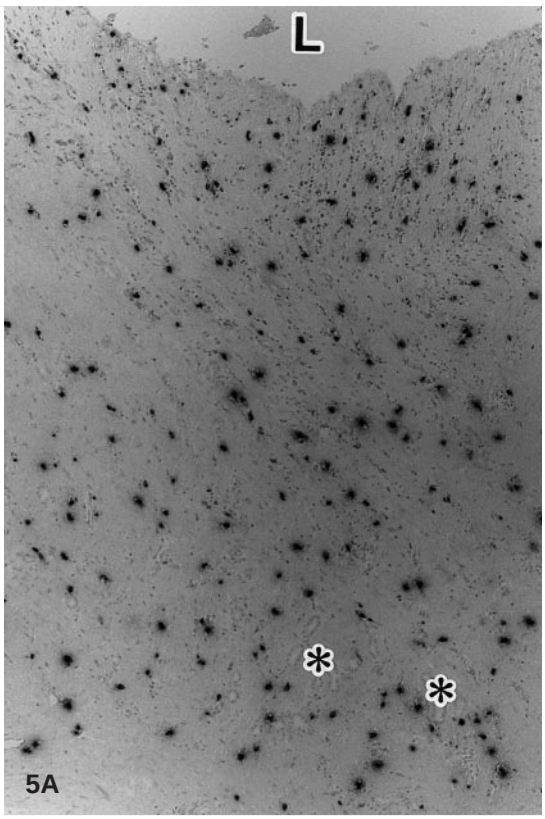
For immunoelectron microscopic examination, fresh fragments of two histologically normal livers and two PBC livers obtained at biopsy were examined by immunoelectron microscopy for histamine and bFGF by the postembedding method [32]. These liver specimens were dissected into small cubes and fixed with 1% glutaraldehyde. The tissues were then dehydrated through a graded ethanol series and embedded in Lowicryl K4M (Chemische Werke

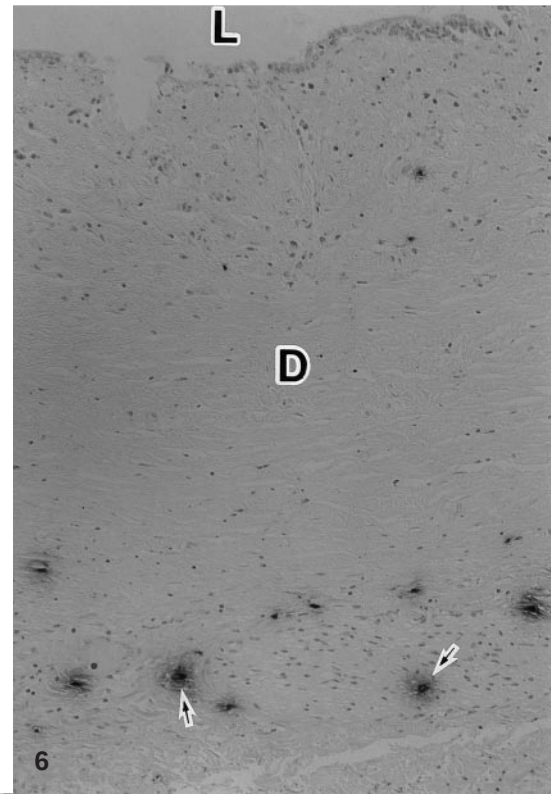
Low, Maldkraiburg, Germany). The tissues were polymerized in an ultraviolet radiation chamber. Semithin sections (1 µm) were stained with toluidine blue to select appropriate areas harbouring the bile ducts from which ultrathin sections (80–100 nm) were mounted on carbon-coated nickel grids and immersed in 1% bovine serum albumin. The sections were incubated in a drop of primary antibodies to histamine or bFGF (Table 2) (diluted 1:2000), then incubated in a drop of secondary antibodies (diluted 1:100) and finally allowed to react with 20 nm protein A-gold particles (E-Y Lab, San Mateo, USA) for 30 min at room temperature.











They were then fixed with 1% osmium tetraoxide, stained with uranium and lead and examined under an electron microscope (H-300; Hitachi, Tokyo, Japan).

Negative controls for immunostaining were performed by substituting the primary antibodies with nonimmune serum, by substituting the secondary antibodies with nonimmune serum, or by omitting protein-A gold for the immunohistochemistry and immunoelectron microscopy.

MCs in hepatic sinusoids were referred to as sinusoidal MCs (Fig. 1A), those in portal tracts and fibrous septa as portal MCs (Fig. 1B) and those around the intrahepatic large bile ducts and their finer branches (septal bile ducts) and intrahepatic peribiliary glands [26] as peribiliary MCs (Fig. 2A, B). MCs were counted by a blind observer as follows, and the mean and standard deviations (SD) were compared among various hepatobiliary diseases. Sinusoidal MCs were counted in more than 10 areas chosen at random in zones 2 and 3 of the hepatic acinus in individual cases (biopsied, surgically resected or autopsy cases, Table 3) under a moderate magnification (×200). Portal MCs were also counted in more than 7 small portal tracts in individual cases (surgically resected, wedge biopsied or autopsy cases; Table 4) at a moderate magnification (×200). Peribiliary MCs were evaluated semiquantitatively in surgically resected or autopsy specimens as ''decreased'', ''normal'', or ''increased'' in comparison with the number of their normal equivalents.

Unless otherwise indicated, all data are expressed as the mean±SD of groups of at least 8 cases. The difference between two group values was tested by Student's unpaired *t*-test, and the difference was considered significant when *P*-values were less than 0.05.

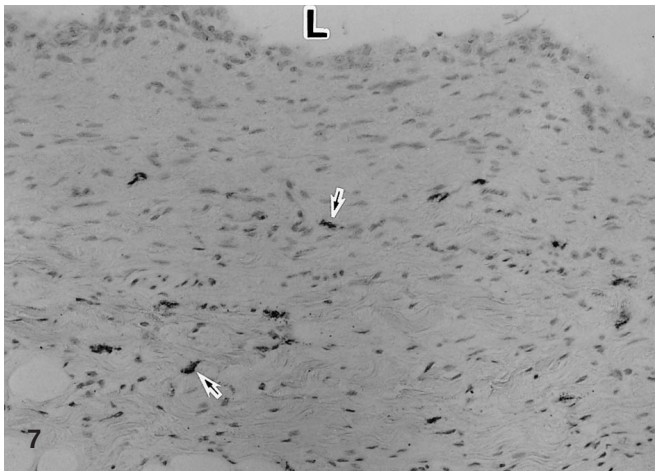
Results

MCs in zones 2 and 3 of the hepatic acinus or in central and middle parts of individual regenerative nodules were not increased in number in either acute or chronic hepatobiliary diseases compared with normal livers (Table 3).

MCs were increased in fibrously enlarged portal tracts in chronic viral hepatitis and alcoholic fibrosis (Table 4, Fig. 3), while they were not increased significantly in tracts with no or minimal fibrosis or variable inflammation [the mean±SD of portal MCs in 3 cases (F_0) and 30 cases ($F_{1–3}$) of chronic viral hepatitis was 1.70±1.30 and

Table 3 Number of mast cells in hepatic lobules (zones 2 and 3) and in cirrhotic regenerative nodules (middle and central parts of individual nodules) in normal livers and various hepatobiliary diseases

Type of liver (no. of cases examined)	No. of mast cells per medium-power field mean±SD
Histologically ''normal livers'' (28)	0.58±0.51
Acute viral hepatitis (19)	0.52±0.59
Acute drug-induced hepatitis (15)	0.44±0.27
Chronic viral hepatitis	
Noncirrhotic (10)	0.39±0.31
Cirrhotic (8)	0.39±0.33
Alcoholic liver fibrosis (8)	1.00±1.23
Primary biliary cirrhosis	
Stage I+II (20)	0.48±0.22
Stage III+IV (7)	0.69±0.29
Primary sclerosing cholangitis (16)	0.61±1.13
Hepatolithiasis (8)	1.35±1.30
Extrahepatic biliary obstruction (8)	0.35±0.22



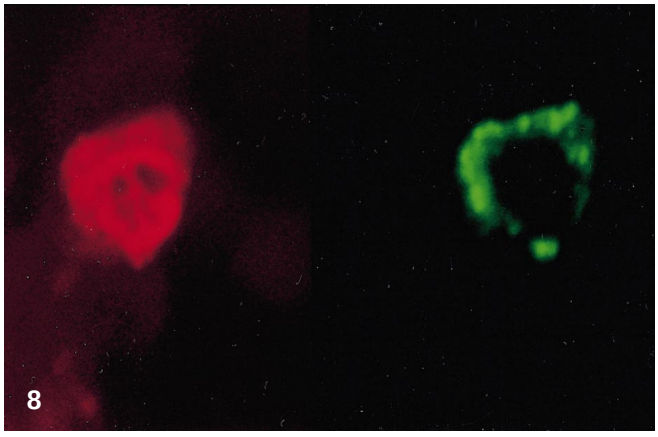


Fig. 7 Mononuclear cells in the fibrotic bile duct are positive for basic fibroblast growth factor (*arrows*). Their distribution and shape suggest that they are peribiliary mast cells (*L* bile duct lumen). Hepatolithiasis. Immunostaining of basic fibroblast growth factor (ABC method) and haematoxylin, ×200

Fig. 8 Double immunostaining of tryptase (*right*, red colour visualized by ABC-alkaline phosphatase and vecta red) and basic fibroblast growth factor (*left*, green colour with fluorescein) in the same mast cell around the bile duct of hepatolithiasis, examined under a confocal laser microscope. ×500

◀ **Fig. 3** Tryptase-positive mast cells are seen in enlarged portal tract showing active inflammation and fibroplasia (*arrows*) and also in the parenchymal sinusoids (*arrowhead*) facing the portal tract (*C* Chronic viral hepatitis, *P* portal tract). Immunostaining of tryptase (ABC method) and haematoxylin, ×350

Fig. 4 Tryptase-positive mast cells (*arrows*) are increased in fibrously enlarged portal tract showing active inflammation (*P* portal tract; *H* hepatic parenchyma). Primary biliary cirrhosis. Immunostaining of tryptase (ABC method) and haematoxylin, ×200

Fig. 5 **A** Numerous tryptase-positive mast cells are seen in the fibrously thickened bile duct of hepatolithiasis. This bile duct shows inflammatory cell infiltration and active fibroplasia (* proliferated peribiliary glands, *L* bile duct lumen). Immunostaining of tryptase (ABC method) and haematoxylin, ×120 **B** Tryptase-positive cells are markedly increased in number of proliferated peribiliary glands (*) of hepatolithiasis. Immunostaining of tryptase (ABC method) and haematoxylin, ×180

Fig. 6 In the sclerotic bile duct wall (*D*) mast cells are absent, while there are tryptase-positive mast cells (*arrows*) in the periductal connective tissue (*L* bile duct lumen). Hepatolithiasis. Immunostaining of tryptase (ABC method) and haematoxylin, ×120

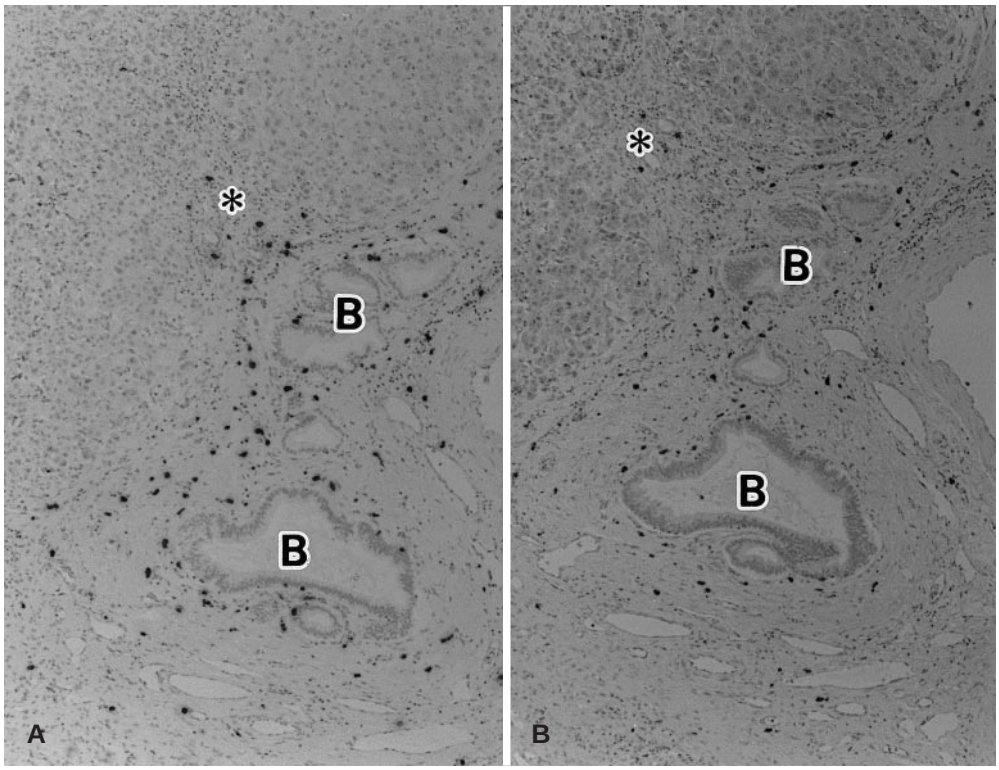


Fig. 9A, B Step serial sections of the same area. Tryptase-positive and histamine-positive cells (*arrows*) are seen in a fibrously enlarged portal tract (*) and around the septal bile ducts (*B*) of primary biliary cirrhosis. More than half of the tryptase-positive cells are also positive for histamine. **A** immunostaining of tryptase (ABC method) and haematoxylin, ×100 **B**, immunostaining of histamine (ABC method) and haematoxylin

Table 4 Number of mast cells in small portal tracts in normal livers and various hepatobiliary diseases

Type of liver (no of cases examined)	No. of mast cells per medium-power field	
	Mean±SD	Statistical difference
Histologically ‘‘normal livers’’ (28)	1.20±0.13	
Acute viral hepatitis (19)	1.26±0.58	*
Acute drug-induced hepatitis (15)	1.36±0.56	
Chronic viral hepatitis		
non-cirrhotic (33)	2.25±1.06	
cirrhotic (16)	2.80±1.23	
Alcoholic liver fibrosis (8)	3.42±4.91	*
Primary biliary cirrhosis		*
Stage I+II (20)	4.93±3.85	
Stage III+IV (7)	8.13±3.11	*
Primary sclerosing cholangitis (16)	3.23±1.55	
Hepatolithiasis (8)	5.08±2.00	
Extrahepatic biliary obstruction (8)	2.49±1.00	

*, $P<0.05$

2.30±1.83]. In biliary/cholestatic diseases, MCs were increased variably and unevently in both fibrotic portal tracts and fibrous septa showing inflammation, but were rare or absent in scarred portal tracts and fibrous septa (Fig. 4). More portal MCs were present in PBC and hepatolithiasis than in chronic viral hepatitis, viral cirrhosis and alcoholic fibrosis. EBO also showed mildly increased portal MCs. MCs were also increaed at the limiting plates and adjoining sinusoids in chronic liver diseases and also in biliary/cholestatic diseases. In contrast, in acute viral and drug-induced liver diseases, MCs in portal tracts were not increased significantly.

Peribiliary MCs were variably increased in the periductal tissue, and to a lesser degree in the ductal walls of the septal and intrahepatic large bile ducts in the biliary diseases, particularly PSC and hepatolithiasis (Fig. 5A). MCs were also increased in the peribiliary glands in these diseases (Fig. 5B). This increase was more evident in the actively fibrotic and inflammatory bile ducts, while the peribiliary MCs decreased in number of disappeared in the sclerotic bile duct walls (Fig. 6).

Sinusoidal and portal MCs and the majority of peribiliary MCs were positive for chymase, another specific marker of tryptase. Some of the peribiliary MCs beneath the biliary lining epithelia were negative for chymase.

b-FGF was inconsistently detected in mononuclear cells in the fibrotic portal tracts and fibrous septa, and to a lesser degree around the septal bile ducts, intrahepatic large bile ducts and peribiliary glands (Fig. 7). Immunostaining of bFGF and tryptase using serial sections and double staining of tryptase and bFGF suggested that some of the tryptase-positive MCs were positive for bFGF (Fig. 8). Around the septal and intrahepatic large bile ducts and peribiliary glands, tryptase-positive MCs beneath the biliary epithelia were negative for bFGF, while those in the ductal wall and periductal fibrous tissue were positive for bFGF (Fig. 7).

Histamine-positive mononuclear cells were scattered in the sinusoids and fibrotic portal tracts, and also around the septal and intrahepatic large bile ducts and peribiliary glands (Fig. 9A, B). These cells resembled sinusoidal, portal, and peribiliary MCs in shape and distribution. Comparison of tryptase and histamine immunostaining in serial sections suggested that more than half of the tryptase-positive MCs were also positive for histamine.

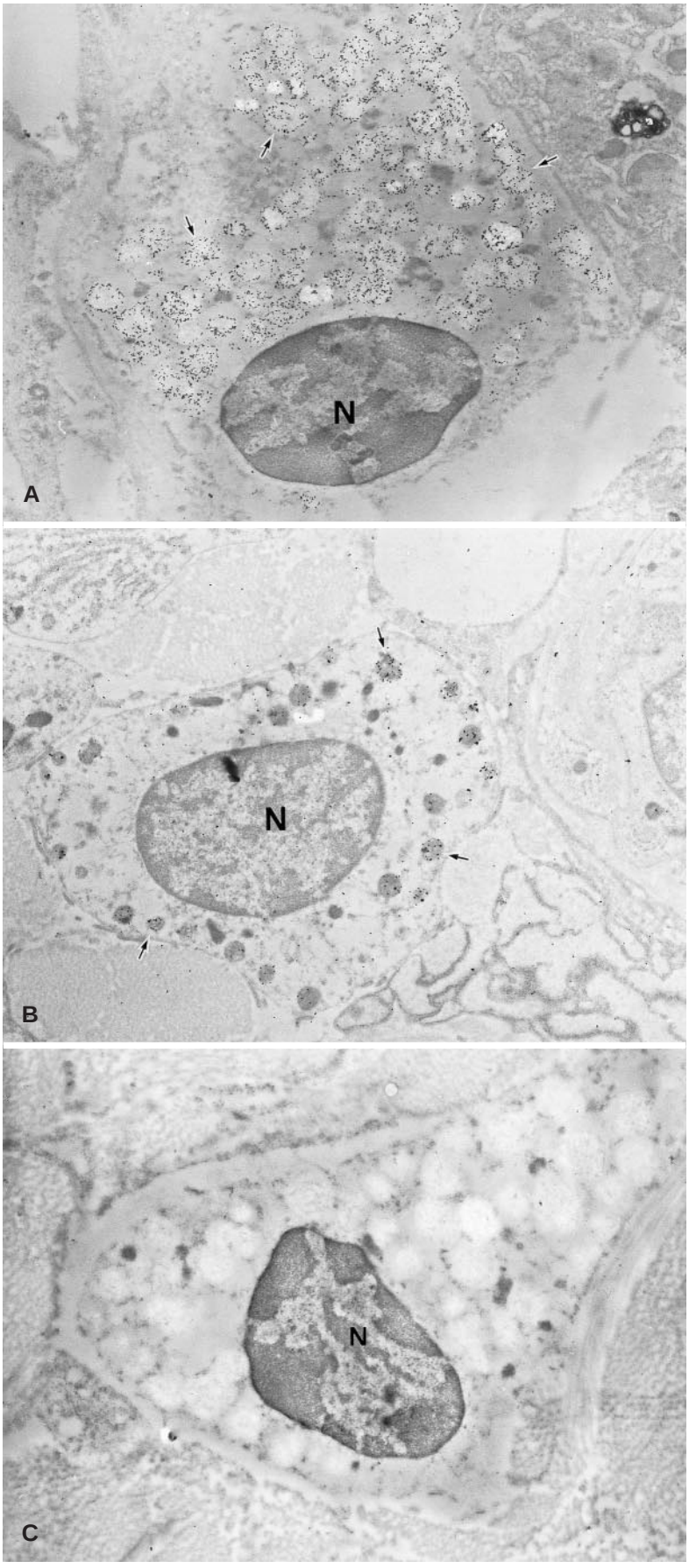


Fig. 10 **A** Gold particles representing basic fibroblast growth factor densely packed in almost all cytoplasmic granules (*arrows*) of a mast cell (*N* nucleus). Primary biliary cirrhosis. Immunoelectron microscopic observation using postembedding and immune gold method, ×12,000 **B** Gold particles representing histamine packed in many granules (*arrows*) of a mast cell (*N* nucleus). Primary biliary cirrhosis. Immunoelectron microscopic observation using postembedding and immune gold method, ×14,000 **C** Gold particles are not detected in the cytoplasm of a mast cell, including the granules (*N* nucleus of a mast cell). Primary biliary cirrhosis. Immunoelectron microscopic observation using post-embedding and immune gold method without primary antibodies (negative control), ×14,500

Immunoelectron microscopy revealed gold particles representing bFGF and histamine in some characteristic granules of MCs (Fig. 10A, B). Immunoreactivity for histamine and bFGF were occasionally detected on the thickened basement membrane around the bile ducts and peribiliary glands and blood vessels. Negative controls for immunostaining consistently resulted in no staining (Fig. 10C).

Discussion

We found that tryptase-positive MCs, which were also largely positive for chymase, were increased in both fibrously enlarged portal tracts and septa in chronic viral hepatitis, cirrhosis, alcoholic fibrosis and cholestatic/biliary diseases. They were not significantly increased in nonfibrotic portal tracts with inflammation without fibrosis in chronic viral hepatitis or in the portal tracts of acute liver diseases. Kollinger et al. [24] recently reported that MCs were increased in the fibrotic portal tract in chronic hepatitis C. This study also disclosed that MCs were markedly increased around the intrahepatic large and septal bile ducts and peribiliary glands showing active fibrosis, cholangitis and/or adenitis [26]. These findings suggest that the increased numbers of portal and peribiliary MCs are incolved in active fibroplasia in these locations.

MCs have been shown to promote fibroblast proliferation, presumably through secretion of fibrogenic mediators. That is, bFGF mRNA and protein were actually detectable in the cytoplasmic granules of human MCs of the skin and lungs [19, 29, 30]. b-FGF has multiple roles in paracrine regulation, such as the stimulation of fibroblast proliferation, remodelling and angiogenesis [10, 11]. In the hepatobiliary system, direct linkage of bFGF to both fibroplasia and repair was recently reported in rat liver injuries [18, 25], but its cellular localization remains unknown. It was shown in this study that the MCs accumulated in the fibrotic portal tracts and fibrous septa and those around the fibrotic biliary tree were positive for bFGF. The gold particles representing bFGF were found by immunoelectron microscopy in the characteristic granules of MCs, suggesting that bFGF is actually stored in these granules. Tryptase is also known to be a potent mitogen for fibroblasts: Ruoss et al. [33] disclosed that low levels of tryptase markedly potentiate DNA synthesis of fibroblasts stimulated by bFGF, suggesting that bFGF and tryptase play a cooperative part in fibrosis. In addition, increased MCs were also positive for histamine both on immunohistochemistry and on immunoelectron microscopy. MC-derived histamine is known to enhance the biosynthesis of collagen by fibroblasts [17, 34], and increase histamine levels in the liver are known to accompany hepatic fibrogenesis [13, 38]. It therefore seems likely that MCs displaying tryptase, bFGF and histamine, are responsible for fibrosis in the above locations in hepatobiliary diseases. More biochemical and in situ information is vital, however, to clarify how these MC-derived mediators are involved in hepatic fibrogenesis.

The reason(s) why portal and peribiliary MCs are more dense in cholestatic/biliary diseases may be related to the leakage or accumulation of biliary substances, including bile acids, which are known to stimulate MCs [8, 28]. Stem cell factor, which is known to stimulate c-kit-expressing MCs, has recently been shown to be expressed on biliary epithelial cells in bile duct-ligated rats [27, 39]. This could also be the case for human biliary/cholestatic diseases.

In contrast, in the acute liver diseases MCs were not increased in the hepatic parenchyma. The present study also failed to show an increase of MCs in foci of pericellular and perivenular fibrosis in the zone 2 and 3 areas of hepatic acinus in chronic liver diseases and cholestatic/biliary diseases, suggesting that MCs are not involved in fibrotic processes within the hepatic parenchyma, where stellate cells may be the main effector cells of liver fibrogenesis [23]. MCs were, however, found in the periportal sinusoids adjoining the destructive and inflamed limiting plates in chronic liver diseases and biliary/cholestatic diseases, suggesting that these MCs are at least involved in periportal fibrosis and inflammation.

We suggest that in chronic hepatobiliary diseases, and in particular in cholestatic/biliary diseases, MCs increase in number and participate in fibrous portal elargement, fibrous septa formation and bile duct fibrosis by producing fibrogenic mediators.

References

1. Amburst T, Batusic D, Ringe B, Ramadori G (1997) Mast cells distribution in human liver disease and experimental rat liver fibrosis. Indications for mast cell participation in development of liver fibrosis. J Hepatol 26:1042–1054
2. Aoki J, Nanba K, Yamamoto T, Sasaki N, Taniyama K (1996) Multiple immunostaining after hot water treatment. Byori to Rinsho 14:1533–1536
3. Bladding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Muller R, Heusser CH, Howarth PH, Holgate ST (1994) Interleukin-4, 5 and 6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 10:471–480
4. Burd PR, Rogers HW, Gordon JR, Martin CA, Jayaraman S, Silson SD, Dvorak AM, Galli SJ, Dorf ME (1989) Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. J Exp Med 170:245–257
5. Celasun B, Crow J, Scheuer PJ (1992) Mast cells in granulomatous diseases. Pathol Res Pract 188:97–100
6. Charlotte F, Win KM, Preaux AM, Mavier P, Dhumeaux D, Zafrani E, Rosenbaum J (1993) Immunolocalization of heparin-binding growth factor (HBGF) types 1 and 2 in rat liver selective hyperexpression of HBGF-2 in carbon tetrachloride-induced fibrosis. J Pathol (Lond) 169:471–476
7. Desmet VJ, Gerber MA, Hoofnagle JH, Manns M, Scheuer PJ (1994) Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 19:1513–1520
8. Farrell DJ, Jones JE, Walls AF, Kelly PJ, Bennett MK, Burt AD (1995) Intrahepatic mast cells in chronic liver diseases. Hepatology 22:1175–1181

9. Feldmann MJ, Morris GP, Dinda PK, Paterson EG (1996) Mast cells mediate acid-induced augmentation of opossum esophageal blood flow via histamine and nitric oxide. Gastroenterology 110:121–128
10. Folkman J, Klagsburn M, Sasse J, Wadzinski M, Ingber D, Vlodavsky I (1988) A herapin-binding angiogenic protein, basic fibroblast growth factor, is stored within basement membrane. Am J Pathol 130:393–400
11. Friedl A, Chang Z, Tierney A, Rapraeger AC (1997) Differential binding of fibroblast growth factor-2 and -7 to basement membrane heparan sulfate. Comparison of normal and abnormal human tissue. Am J Pathol 150:1443–1455
12. Friedman SL (1990) Cellular sources of collagen and regulation of collagen production in liver. Semin Liver Dis 10:20–29
13. Gittlen SD, Schulman ES, Maddrey WC (1990) Raised histamine concentration in chronic cholestatic liver disease. Gut 31:2007–2011
14. Gordon JR, Galli SJ (1990) Mast cells as a source of both preformed and immunological inducible TNF-α/cachectin. Nature 346:274–276
15. Gordon JR, Galli SJ (1994) Promotion of mouse fibroblast collagen gene expression by mast cells stimulated via the FceRI: role for mast cell-derived transforming growth factor-β and tumor necrosis-α. J Exp Med 180:245–257
16. Guidry C, Grinnell F (1987) Heparin modulates the organization of hydrate collagen gels and inhibits gel contraction by fibroblasts. J Cell Biol 104:1097–1103
17. Hatamochi A, Fujiwara K, Ueki H (1985) Effects of histamine on collagen gel synthesis by cultured fibroblasts derived from guinea pig skin. Arch Dermatol Res 277:60–64
18. Hioki O, Minemura M, Shimizu Y, Kashii Y, Nishimori H, Takahara T, Higuchi K, Yoshitake Y, Watanabe A (1996) Expression and localization of basic fibroblast growth factor (bFGF) in the repair process of rat liver injury. J Hepatol 24:217–224
19. Inoue Y, King TE, Tinkle SS, Dockstader K, Newman LS (1996) Human mast cell basic fibroblast growth factor in pulmonary fibrotic disorders. Am J Pathol 149:2037–2054
20. Irai AA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB (1989) Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. J Histochem Cytochem 37:1509–1515
21. Jakobsson AE (1994) Angiogenesis induced by mast cell secretion in rat peritoneal connective tissue in a process of three phases. Microvasc Res 47:252–269
22. Jordana M (1993) Mast cells and fibrosis: who's on first? Am J Respir Cell Mol Biol 8:7–8
23. Knittel T, Schuppan D, Meyer zum Buschenfelde KH, Ramadori G (1992) Differential expression of collagen types I, III, and IV by fat-storing (Ito cells) in vivo. Gastroenterology 102:1724–1735
24. Kollinger M, Gelbmann CM, Kubitza M, Dei LL, Scholmerich J, Holstege A (1997) Distribution of mast cells in hepatitis-C infected human livers (abstract). Gastroenterology 112: A1307
25. Muller D, Enderle GJ, Low O, Dietze E, Krell H (1996) Bile ductular proliferation and altered leukotriene elimination in thioacetamine-induced fibrosis of rat liver. J Hepatol 25: 547–553
26. Nakanuma Y, Hoso M, Sanzen T, Saski M (1997) Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. Microsc Res Tech 38: 552–570
27. Omori M, Evarts RP, Omori N, Hu Z, Marsden ER, Thorgeirsson SS (1997) Expression of α-fetoprotein and stem cell factor/c-kit young rats. Hepatology 25:1115–1122
28. O'Rourke D, Clements B, Ennis M, Erwin P, Camphell G, Halliday HI, Rowlands BJ (1993) Mast cell activation in obstructive jaundice (abstract). J Pathol (Lond) [Suppl]:398A
29. Qu Z, Liebler JM, Powers MR, Galey T, Ahmadi P, Huang XN, Ansel JC, Butlerfield JH, Planck SR, Risebbayn JT (1995) Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. Am J Pathol 47:564–573
30. Reed JA, Albino AP, McNutt NS (1995) Human cutaneous mat cells express basic fibroblast growth factor. Lab Invest 72: 215–222
31. Rioux KP, Sharkey KA, Wallace JL, Swain MG (1996) Hepatic mucosal mast cell hyperplasia in rats with secondary biliary cirrhosis. Hepatology 23:888–895
32. Roth J (1982) The preparation of protein A-gold complexes with 3 nm and 15 nm gold particles and their use in labeling multiple antigens on ultrathin sections. Histochem J 14: 791–801
33. Ruoss SJ, Hartmann T, Caughey GH (1991) Mast cell tryptase is a mitogen for cultured fibroblasts. J Clin Invest 88:493–489
34. Russell JD, Russell SB, Trupin KM (1977) The effect of histamine on the growth of cultured fibroblasts isolated from normal and keloid tissue. J Cell Physiol 93:389–393
35. Sato Y, Mukai K, Watanabe S, Goto M, Shimosato Y (1986) The AMeX method: a simplified technique of tissue processing and paraffin embedding with improved preservation of antigens for immuno-staining. Am J Pathol 125:431–435
36. Scheuer PJ (1980) Liver biopsy interpretation, 3rd edn. Baillière-Tindall, London
37. Thompson HL, Burkelo PD, Babreil G, Yamada Y, Metclfe DD (1991) Murine mast cells synthesize basement membrane components. A potential role in early fibrosis. J Clin Invest 87:619–623
38. Umezu K, Yuasa S, Sudoh A (1990) Change of hepatic histamine concentrations in chronic cholestatic liver disease. Gut 31:96–99
39. Valent P, Spanblochl E, Sperr WR, Sillaber C, Zsebo KM, Agis H, Strobl H, Geissler K, Bettleheim P, Lechner K (1992) Induction of differentiation of human mast cells from bone marrow and peripheral blood mononuclear cells by recombinant human stell cell factor/kit-ligand in long term culture. Blood 80:2237–2245