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Role of mast cells in hepatic remodeling during cholestasis and its resolution: relevance to regulation of apoptosis

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Abstract

Background/aims: Mast cells are thought to be related to fibrogenesis, but recent studies have shown that fibrosis of the liver can be induced even in mast cell-deficient rats. To clarify the significance of mast cell accumulation in cholestatic liver diseases, the relations between such accumulation, bile ductule proliferation and apoptosis of biliary epithelial cells were examined in the rats during cholestasis and its resolution.

Methods: Cholestasis and its resolution were induced in rats by common bile duct ligation and spontaneous recanalization, respectively. The extent of bile ductule proliferation and the numbers of mast cells and apoptotic biliary epithelial cells were estimated quantitatively in liver sections.

Results: Recanalization of the ligated common bile duct led to an abrupt and transient increase in the number of mast cells, although the number of proliferated bile ductules decreased rapidly. The number of apoptotic biliary epithelial cells of the proliferated bile ductules increased rapidly and transiently, and the change paralleled that of the mast cells.

Conclusions: Mast cells accumulating in the portal triads during cholestasis and its resolution may relate to the reduction of proliferated bile ductules, i.e., in hepatic remodeling, through the induction of apoptosis of biliary epithelial cells.

Keywords: Mast cell; Hepatic remodeling; Apoptosis; Cholestasis; Common bile duct ligation; Liver fibrosis; Bile ductule proliferation

Introduction

Activated mast cells are thought to secrete a large number of fibrogenic factors and be involved in the development of fibrosis in various tissues and organs (Liebler et al., 1998; Abe et al., 1998; Pardo et al., 2000; Roberts and Brenchley, 2000; Kondo et al., 2001). In the liver, in particular, mast cell accumulation is reportedly seen in association with alcoholic liver disease (Farrell et al., 1995; Matsunaga et al., 1999), primary biliary cirrhosis (Farrell et al., 1995; Matsunaga et al., 1999), primary sclerosing cholangitis (Tsuneyama et al., 1999), biliary atresia (Uddin-Ahmed et al., 2000), hepatolithiasis (Tsuneyama et al., 1999), chronic hepatic allograft rejection (O'Keeffe et al., 2002) and cholestatic biliary disease (Yamashiro et al., 1998). The mast cells secrete various mediators, promoting fibroblast growth, and stimulate production of the extracellular matrix by fibroblasts or stellate cells. In fact, there is a positive correlation between the degree of mast cell accumulation and the extent of matrix deposition in patients with

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chronic liver disease (Farrell et al., 1995). To the contrary, some recent studies have shown that bile duct ligation or administration of carbon tetrachloride can induce liver fibrosis even in mast cell-deficient rats and that the magnitude of fibrosis is significantly greater in these rats than in normal rats (Okazaki et al., 1998; Sugihara et al., 1999). The implication is that mast cells are not necessary for development of fibrosis, and consequently the role of mast cells in the liver is not clear at present. However, it is a hard fact that mast cell accumulation in the liver occurs with various liver diseases. We believe that mast cells may relate to the increase and decrease in bile ductules, i.e. in hepatic remodeling, through induction of apoptosis, because mast cell accumulation appears together with bile ductule proliferation, especially in cholestatic liver diseases. To clarify the significance of mast cell accumulation in the liver, we examined the relations between mast cell accumulation, hepatic remodeling, i.e. proliferation and reduction of bile ductules and apoptosis of biliary epithelial cells during cholestasis and its resolution in rats.

Materials and methods

Two hundred and twenty-five male Wistar rats weighing about 100 g were maintained in community wire-woven cages at five animals per cage under a 12–12 light–dark cycle and 22–24 °C. The animals were housed for 1-week prior to the experiments and maintained on a standard pellet diet and tap water ad libitum. Surgery was performed via an upper midline incision under light ether anesthesia. In 200 rats, the common bile duct at the liver hilum was tied in one place. In sham-operated rats (25 rats), a silk ligature was placed around the common bile duct and then removed. The level of bilirubin in urine was examined every day after the operation to evaluate whether the ligated common bile duct recanalized: in about 10% of the rats with common bile duct ligation, recanalization occurred 14 days after the operation. The abdomen was reopened 3, 4, 7 or 14 days after recanalization in these rats and at 4, 10, 14, 21 or 28 days after the operation in the rats without recanalization (five rats in each group). Blood was taken from the inferior vena cava for liver function tests. The liver was extirpated for histological examination after the animal was killed.

For light microscopic observation, the livers were fixed in 10% neutral buffered formalin for 18 h and embedded in paraffin, and sections were stained with hematoxylin and eosin, by Gomori's method for reticulum or by Mallory's method for collagen. For visualizing and measuring mast cells, sections were stained with toluidine blue (pH 4.1). The number of mast cells was measured under the microscope with the use of a square micrometer eyepiece. A 200-fold magnification was used, and 50 fields from each liver were scanned. Values were expressed as the number of mast cells per mm^2 of liver section and of portal triad.

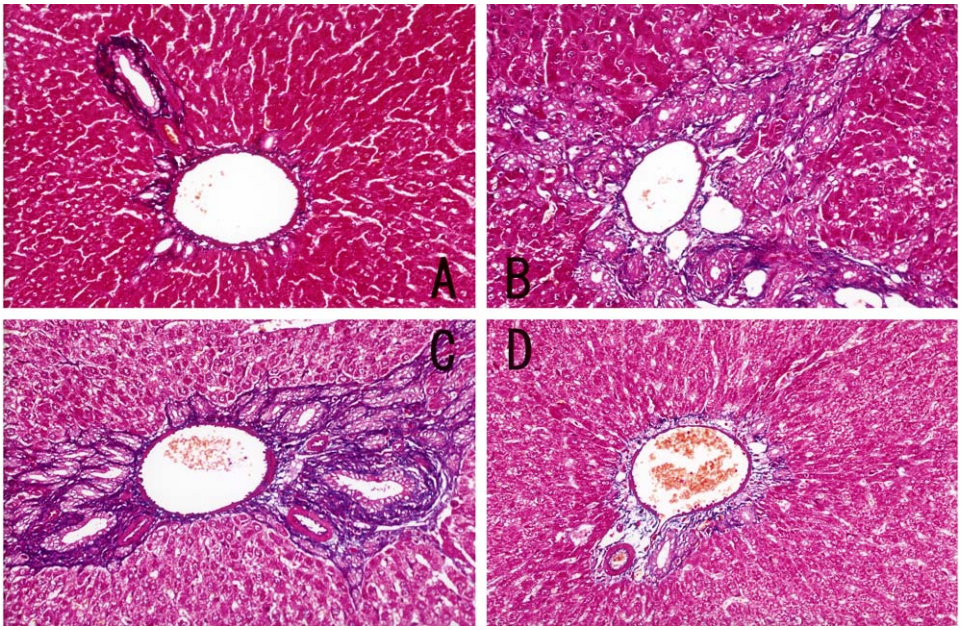

Fig. 1. Changes in the portal triad visualized by Mallory's method for collagen × 200. (A) Liver of a sham-operated rat. There is no remarkable change. (B) Liver of a rat 14 days after common bile ligation. The portal triads are expanded due to proliferation of bile ductules with deposition of loose collagen fibers. (C) Liver of a rat 3 days after recanalization of the common bile duct. The extent of proliferation of bile ductules is the same as in (B). (D) Liver of a rat 14 days after recanalization of the common bile duct. Proliferated bile ductules and collagen fibers in the portal triads have disappeared.