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Case Report

Macrophages, myofibroblasts and mast cells in a rat liver infected with *Capillaria hepatica*

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We trapped a rat (*Rattus norvegicus*) infected with *Capillaria hepatica*. At necropsy, grossly yellowish-white nodules (2-3 mm in diameter) were noted to be scattered on the liver's surface. Microscopically, granulomatous and fibrotic nodules that contained the eggs and/or adult worms of *Capillaria hepatica* were detected in the liver. Septal fibrosis was diffusely formed throughout the liver. There were a number of ED1-positive macrophages located in the sinusoids of the pseudolobules. On the double staining, myofibroblasts and mast cells were generally observed within the fibrous septa with the mast cells in close proximity to the myofibroblasts. We suggest that the interactions between macrophages, myofibroblasts and mast cells play a role in the septal fibrosis observed in rats infected by *Capillaria hepatica*.

Keywords: *Capillaria hepatica*, fibrosis, liver, rat

Capillaria (*C.*) *hepatica* is a zoonotic liver nematode of mammals and this disease has a global distribution [1]. *C. hepatica* is found primarily in rodents, although it has been reported in other animals and humans as well [1,2]. As the parasite's name implies, the worms live in the host's liver. The female produces eggs in the liver parenchyma and all the worms die inside the liver around the 30th to 40th day after infection [3]. Breakdown of the dead worms and lease of eggs cause focal necrosis and encapsulation. When all the worms are dead and being disintegrated, the focal encapsulating fibrous response eventually progresses to septal fibrosis, and cirrhosis has also been reported [3].

A feral rat (*Rattus norvegicus*) was trapped and necropsied. For histopathological observations, pieces of its liver were immediately fixed in 10% neutral buffered formalin; they were then routinely processed and embedded in paraffin. Tissue sections 4 μm in thickness were cut and stained with hematoxylin and eosin and toluidine blue, and Azan stain was used for staining the collagen. For immunohistochemistry, sections of liver were deparaffinized in xylene, rehydrated in a series of graded alcohol solutions and then incubated in a solution of 3% hydrogen peroxide in methanol for 10 min. The tissue sections were washed with PBS and then immunostained with the primary antibodies, anti-rat monocyte/macrophage at a dilution of 1 : 600 (clone ED-1; Chemicon, USA) and monoclonal anti-α-smooth muscle actin (α-SMA) at a dilution of 1 : 800 (clone 1A4; Sigma, USA). The antigen-antibody complex was visualized by the labelled streptavidin-biotin (SAB) method and using a Histostatin-plus bulk kit (Zymed Laboratories, USA) with 3,3-diamino-benzidine (Zymed Laboratories, USA). Non-immunized goat sera, which were used instead of the primary antibody, served as the negative control. The tissue sections were then rinsed in distilled water and counterstained with Mayer's hematoxylin or toluidine blue for simultaneous observation of the myofibroblasts and mast cells in the liver.

At necropsy, grossly yellowish-white nodules containing the eggs and/or the adult worms of *C. hepatica* were readily identified microscopically as granulomas, and these were scattered throughout the liver (Fig. 1). Both viable and degenerate adult nematodes and eggs were present. Some necrotic worms were mineralized. Others appeared focally accumulated, and these formed discrete inflammatory nodules surrounded by connective tissue, macrophages, eosinophils, lymphocytes and plasma cells. Increased numbers of mast cells were detected around the central veins, portal areas and fibrous septa. Clusters of eggs and worms were surrounded by collagen fibers. Septal fibrosis that formed pseudolobules was observed as a diffuse change throughout the liver. Even in areas unassociated with granulomas,

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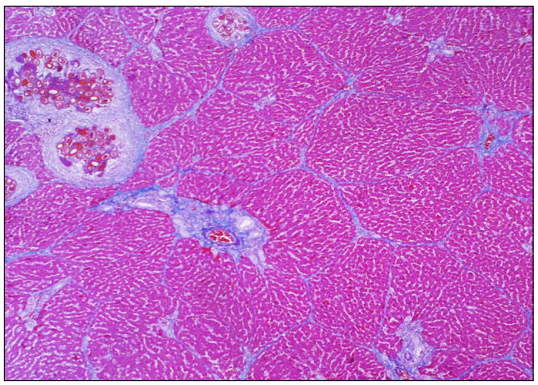

Fig. 1. Collagen fibers are detected in the portal areas and note the fibrous septa that form pseudolobules. Azan stain. ×13.

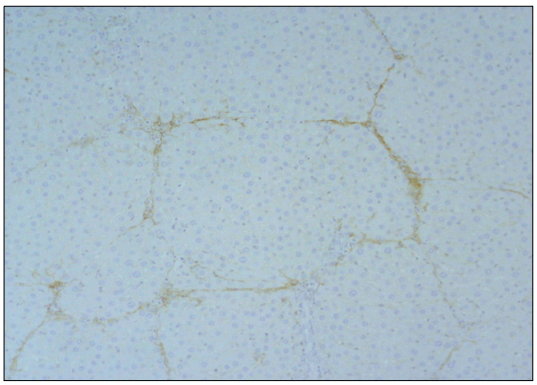

Fig. 3. α-SMA positive myofibroblasts are observed in the fibrous septa and they form pseudolobules. SAB method. ×33.

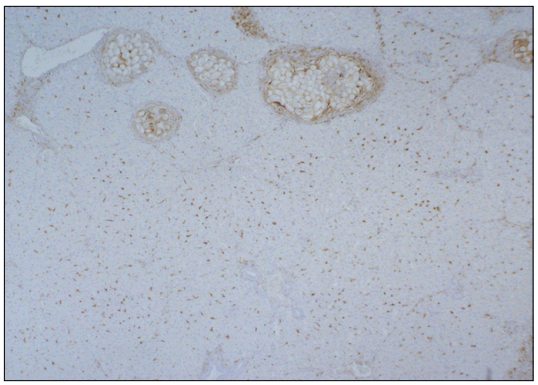

Fig. 2. There are a number of ED1-positive macrophages located in the sinusoids of the pseudolobules. SAB method. ×13.

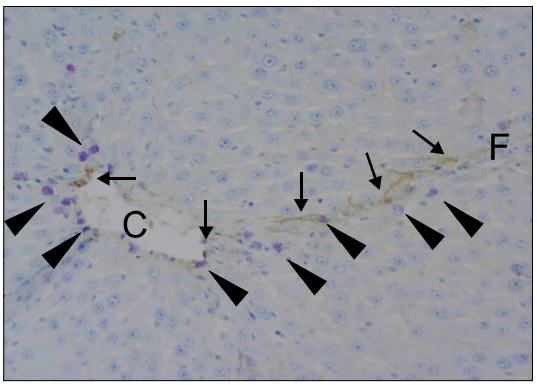

Fig. 4. Mast cells (arrow head) and myofibroblasts (arrow) are simultaneously detected within the fibrous septa (F) and around the central vein (C). Mast cells are largely observed along the myofibroblasts. SAB method and toluidine blue stain. ×66.

delicate fibrous septa bridged between the central veins to the portal areas (Fig. 1). There were a number of ED1- positive cells located in the sinusoids of the pseudolobules (Fig. 2). Their morphology suggested that most of them were Kupffer cells. α-SMA positive myofibroblasts were observed within the fibrous septa (Fig. 3) and within the walls of the fibrotic granulomas. They were also detected in the connective tissues surrounding the fibrotic nodules. On double staining, myofibroblasts and mast cells were generally observed within the fibrous septa with mast cells being in close proximity to the myofibroblasts (Fig. 4).

Septal fibrosis is a commonly seen variant of hepatic fibrosis, and this is represented by thin, straight fibrosis tissue septa that dissect the liver parenchyma and sometimes circumscribe the hepatocellular nodules in cirrhotic livers [4]. Septal fibrosis of the liver can be experimentally produced in rats by intraperitoneal injection of porcine serum or *C. hepatica* infection [5], although its etiology and pathogenesis are poorly understood [1,6,7]. The pathogenesis of septal fibrosis associated with *C. hepatica* might be related to the slow release of sequestered antigenic materials from the dead worms and eggs [3,5]. The feature of septal fibrosis in *C. hepatica* infected rats is similar to that seen in rats repeatedly injected with porcine serum. Its etiology and pathogenesis are poorly understood. The pathogenesis of septal fibrosis has been considered to have an immunological basis in both cases [6,8].

The resident macrophages of the liver, i.e., the Kupffer cells, normally protect the hepatocytes by phagocytosing any incoming particles. However, in the liver, xenobiotics induce localized Kupffer cell accumulation and the release of mediators, which have the potential to kill the hepatocytes [9]. It has been recently reported that myofibroblasts (activated hepatic stellate cells) are a major producer of the extracellular matrix and the myofibroblasts play a pivotal role in liver fibrosis [10]. Furthermore, many studies have reported that macrophages, myofibroblasts and mast cells are closely related to the liver fibrosis induced by CCl_4 [10,11] and porcine serum [1,6]. Our previous report demonstrated that the number of macrophages, myofi-

broblasts and mast cells were increased in animals with hepatic fibrosis and these cells might play a role in the formation of hepatic fibrosis [5]. However, no previous studies have reported on the relationship and role of macrophages, myofibroblasts and mast cells in the hepatic septal fibrosis induced by *C. hepatica*. We hypothesize that a similar mechanism involving macrophages, myo-fibroblasts and mast cells might be associated with the septal fibrosis in this rat infected with *C. hepatica*. Moreover, by double staining via immunohistochemistry and the toluidine blue stain method, we observed the close proximity of myofibroblasts and mast cells in the liver in the hepatic fibrous septa of an animal infected with *C. hepatica*. This finding is consistent with that previously seen in a rat model of CCl_4-induced hepatic fibrosis; Akiyoshi *et al.* [11] reported that in this rat model, the myo-fibroblasts were closely associated with mast cells, and nerves and mast cells were largely observed along the myofibroblasts. As a possible mechanism for cirrhosis [12], the relationship between the development of myofibroblasts and mast cell-released fibrogenic cytokines (platelet-derived growth factor and transforming growth factor-beta) might be involved in the progress of hepatic inflammation, fibrosis and cirrhosis in animals with *C. hepatica* infection. Our observations closely parallel their findings. Thus, we believe that macrophages, myo-fibroblasts and mast cells might play a role in the septal fibrosis induced by *C. hepatica* infection in rat liver.

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