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The role and immunophenotypic characteristics of myofibroblasts in liver of sheep naturally infected with the lancet liver fluke (*Dicrocoelium dendriticum*)

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ABSTRACT

The main objective of our research was to examine the role and immunophenotypic characteristics of myofibroblasts in sheep liver naturally infected by the lancet liver fluke (*Dicrocoelium dendriticum*). In the reported study we analyzed liver samples from 20 adult sheep, 14 infected animals and 6 controls. The liver samples were fixed in 10% buffered formalin, and routinely processed and stained using hematoxylin eosin, the periodic acid-Schiff and Masson–Goldner trichrome methods. The immunohistochemical examination was carried out by the streptavidin biotin (LSAB2) method, using antibodies for α-smooth muscle actin (α-SMA), desmin and vimentin. The histopathological examination revealed liver fibrosis in 6 out of 14 (42.9%) analyzed samples, while different forms of cholangitis were observed in the remaining 8 out of 14 (57.1%). The expression of α-SMA was proven in perisinusoidal hepatic stellate cells, portal/septal myofibroblasts, and interface myofibroblasts. The degree of α-SMA expression and the number of α-SMA immunopositive cells were the most intensive in the liver with fibrosis. Desmin expression in all liver samples of infected sheep was confirmed in hepatic stellate cells and smooth muscle cells. The hepatic stellate cells, portal/septal myofibroblasts, and interface myofibroblasts reacted as vimentin positive cells. In the liver without fibrotic changes hepatic stellate cells and smooth muscle cells were desmin positive. The obtained results suggest that all populations of myofibroblasts, especially hepatic stellate cells, play an important role in the increased extracellular matrix formation during parasitic liver fibrosis in sheep naturally infected with *D. dendriticum*.

1. Introduction

The lancet liver fluke (*Dicrocoelium dendriticum*) lives in the bile ducts of domestic and wild ruminants (sheep, goats, cattle, buffaloes, red deer) and occasionally affects rabbits, pigs, dogs, horses and humans. The development and degree of changes depend on the duration and severity of the infection and the age of the infected animals (Camara et al., 1996; Otranto and Traversa, 2003). In the first few days following infection, angiectasis of the hepatic central veins and the portobiliary vessels are observed. Furthermore, the parasite causes changes in the bile ducts

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in the form of catarrhal and proliferative cholangitis and marked distension of bile ducts. Due to its buccal stiletes, the lancet liver fluke irritates the bile duct surfaces, thus causing proliferation and changes in the septal bile ducts of the lobular hepatic edges. In addition to the mechanical irritation caused by the migrant flukes, pathologic changes have been attributed to the toxic effects of metabolic products released by the parasite. Hyperplasia of bile duct epithelium and periductal inflammation are present in the advanced stages of the disease. In the terminal stage, it leads to the development of biliary fibrosis and liver cirrhosis (Manga-Gonzalez et al., 2004). Different species of parasites can lead to an increase in extracellular matrix and the development of liver fibrosis (Anthony et al., 2010; Golbar et al., 2013).

Chronic liver and bile duct injuries caused by various agents lead to the activation of different cell populations and their transdifferentiation to myofibroblasts (MFs), acquiring contractile, proinflammatory, and fibrogenic properties (Bataller and Brenner, 2005; Lemoinne et al., 2013; Micallef et al., 2012). The largest contribution to extracellular matrix and fibrogenesis probably derives from mesenchymal MFs-like subpopulations of the liver. Activated fibroblasts which develop MFs characteristics play an essential role in hepatic fibrogenesis (Duffield et al., 2013; Forbes and Parola, 2011; Honda et al., 2013). Comprehensive studies have described three different MFs-like cells in rats and humans based on location and immunohistochemical profile. Essentially, MFs comprise (a) portal or septal MFs, present in the portal areas or in newly formed fibrous septa and for the most part come from the portal fibroblasts, (b) interface MFs, present at the interface between parenchyma and stroma of the portal areas or newly formed fibrous septa and according to their antigen profile, probably originate from activated hepatic stellate cells, and (c) the perisinusoidally located hepatic stellate cells (HSCs) originating from dormant inactive HSCs (Brenner et al., 2012; Fausther et al., 2013; Jiang and Torok, 2013; Parola and Pinzani, 2009). Activated MFs by their phenotypic characteristics differ from the cells from which they originate. Morphologically, the ability of contraction is reflected in the fact that MFs express α-smooth muscle actin (α-SMA), contractile protein in their cytoplasm (Hinz et al., 2007; Novo et al., 2014; Rockey et al., 2013).

Activated HSCs in humans, rats, dogs, cats, cattle and fallow deer are the most significant cells that are involved in the synthesis of the extracellular matrix during liver fibrosis (Aleksić-Kovačević et al., 2010; Cassiman et al., 2002; Golbar et al., 2013; Ide et al., 2001; Knežević et al., 2009; Kukolj, 2014; Marinkovic et al., 2013; Mekonnen et al., 2007). Immunophenotypic characteristics of quiet, inactive HSCs vary depending on the animal species. In the normal liver of rats and humans α-smooth muscle actin (α-SMA) expression was not observed (Cassiman et al., 2002; Mallat and Lotersztajn, 2013). HSCs in the normal swine liver are desmin and vimentin positive, but α-SMA negative (Uetsuka et al., 2007). However, HSCs in the liver of healthy dogs and fallow deer are positive for α-SMA (Ijzer et al., 2006; Knežević et al., 2009; Marinkovic et al., 2013). HSCs in the liver of healthy cattle are immunopositive to α-SMA and vimentin and weakly positive to desmin (Golbar et al., 2013).

As a consequence of liver damage, HSCs are activated, and they are transformed from inactive, dormant cells, into MFs-like cells with new phenotype characteristics. The most significant morphological changes in activated HSCs include an increase in the intensity of α-SMA expression, a reduction of GFAP expression and a loss of fat droplets in the cytoplasm (Gressner and Gao, 2014; Guyot et al., 2006; Honda et al., 2013). α-SMA is the most commonly used marker and a reliable indicator of HSCs activation (Bataller and Brenner, 2005; Rockey et al., 2013). Previous studies have demonstrated the correlation between the degree of liver fibrosis and the number of activated HSCs present in the liver (Aleksić-Kovačević et al., 2010; Knežević et al., 2009; Moreira, 2007).

The main objective of this study was to determine the origin of myofibroblasts in fibrotic areas in the liver of sheep naturally infected by the lancet liver fluke.

2. Material and methods

2.1. Animals

Liver tissue of dead sheep was sampled at the Department of Pathology of the Faculty of Veterinary Medicine at the University of Belgrade during the period of 2–12 h following death. A total of 20 adult sheep carcasses, 14 infected animals (11 females and 3 males) and 6 controls were examined. At necropsy, macroscopic changes that correlate to infection with the lancet liver fluke were found in the livers of all 14 infected sheep. During the macroscopic examination, adult forms of the lancet liver fluke were counted, as well as the number of bile ducts containing parasites. The method used for counting the adult forms of *D. dendriticum* was described by Camara et al. (1996). The liver samples obtained from 6 healthy sheep, both sexes (5 females and 1 male), without macroscopic and microscopic changes and evidence of *D. dendriticum* infection, were used as the control samples.

2.2. Parasitological analysis

The collected parasites were isolated and identified by direct microscopical observation with the wet mount technique, based on the morphological and morphometric characteristics of the isolated parasites (Kassai, 1999).

2.3. Histopathological and immunohistochemical analysis

At necropsy, 3–4 liver samples were taken from the left lobe for histological and immunohistochemical analysis.

The liver samples were fixed in 10% buffered formalin, processed in an automated tissue processor and embedded in paraffin blocks. Paraffin sections 3–5 μm thick were routinely stained with hematoxylin and eosin (HE) (Fischer et al., 2008), the periodic acid-Schiff (PAS) (McManus, 1948) and Masson–Goldner trichrome (MTH) (Goldner, 1938) methods.