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Susceptibility of chicken Kupffer cells to Chinese virulent infectious bursal disease virus

Haiyan Ma, Sufen Zhao, Yunfei Ma, Xin Guo, Deping Han, Yuanyuan Jia, Weiwei Zhang, Kedao Teng *

College of Veterinary Medicine, China Agricultural University, Beijing, People's Republic of China

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ABSTRACT

Infectious bursal disease (IBD) is an acute, highly contagious, and immunosuppressive avian disease caused by IBD virus (IBDV). Although the effects of IBDV on bursa of Fabricius in chickens have been well reported, the impacts of IBDV on liver after IBDV infection are still unclear. In the present study, specific pathogen free (SPF) chickens were experimentally inoculated with IBDV Chinese virulent strain BC6/85, and the cells in liver and bursa were examined by immunohistochemisrty and transmission electron microscopy (TEM). The congestion of liver tissue and fatty degeneration of hepatocytes were characteristics of microscopical changes in chicken liver at 3 days post infection (d.p.i.), whereas there were follicular lymphoid necrosis, apoptosis, depletion, as well as edema and congestion in bursa. In addition, the number of IBDV-positive cells peaked at 4 d.p.i. in bursa and at 3 d.p.i. in liver, respectively. With respect to ultrastructural pathological changes of hepatocytes, mitochondria swelled and nucleus deformed into an irregular shape or its chromatin peripherally condensed which indicated that the hepatocyte was at the early stage of apoptosis, and the electron-lucent lipid droplets in a variety of sizes were observed within cytoplasm. Kupffer cells became "swollen-like" and the electron-density of their cytoplasm was lower than that of cells in uninfected group. Liver glycogen deposits significantly declined from 2 to 5 d.p.i. and recovered strongly at 6 d.p.i. More importantly, KLU01 (macrophage marker) positive ($KUL01^+$) cells were infiltrated in bursa and liver in IBDV-exposed chickens by immunoperoxidase staining. To demonstrate the correlation between IBDV and macrophages in bursa and liver, we further investigated the colocalization of viral antigens and macrophages by double immunofluorescence labeling. At 4 d.p.i., the percentage of double positive cells (IBDV positive and $KUL01^+$ cells) accounted for 26.5 percent of the total IBDV positive cells or 57 percent of the total $KUL01^+$ cells in bursa. In comparison, the percentage of double positive cells in liver constituted 97 percent of the total IBDV positive cells or 99 percent of the total $KUL01^+$ cells. These results suggest that IBDV was susceptible to $KUL01^+$ cells in liver (mainly Kupffer cells) and replicated in the $KUL01^+$ cells. By comparison with the influence of IBDV on bursa, our findings were the first to elucidate the pathological changes in liver after IBDV infection on a microscopical and ultrastructural scale, and, especially, to gain the initial insight into the susceptibility of Kupffer cells to IBDV.

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1. Introduction

Infectious bursal disease (IBD) caused by infectious bursal disease virus (IBDV) is a highly contagious and immunosuppressive viral disease in young chickens during

* Corresponding author at: College of Veterinary Medicine, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing 100193, People's Republic of China. Tel.: +86 01 62733013.
E-mail addresses: kedao@cau.edu.cn, mahaiyan.attitude@163.com (K. Teng).

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3 to 6 weeks of age. IBDV infection may aggravate infection with other pathogenic agents, and IBDV-infected chickens have the low ability of response to subsequent vaccinations (Müller et al., 2003). IBDV can be classified into two serotypes (serotype 1 and 2) by virus neutralization test (McFerran et al., 1980). Serotype 1 strains, differentiated as classical strains (cIBDV), very virulent strains (vvIBDV) and variant strains (vIBDV), can cause pathogenicity and mortality in young chickens, while serotype 2 strains are avirulent (Liu et al., 2010; Rauf et al., 2011).

The causative agent, IBDV, belongs to the family Birnaviridae, and is non-enveloped, double-stranded (ds) RNA virus consisting of two segments (segment A and segment B) (Dobos et al., 1979). Segment A encodes the VP5, VP4 (28 kDa), VP3 (32 kDa), and VP2 (41 kDa), and segment B encodes VP1 (98 kDa) (Mundt et al., 1995; Birghan et al., 2000; Von Einem et al., 2004). IBDV infects and destroys actively dividing IgM-bearing B cells in the bursa of Fabricius (Hirai et al., 1981; Rodenberg et al., 1994). Additionally, the extensive viral replication is present in bursa (Dobos et al., 1979). So viral infection causes severe depletion of lymphocytes and destruction of bursa tissues of chickens infected with IBDV (Käufer and Weiss, 1980). Although bursa is the principal target organ for IBDV, recent studies show that the virus also infects and possibly replicates in other organs, such as spleen, thymus, bone marrow, cecal tonsil, liver and kidney, at early stage of infection (Tanimura et al., 1995; Oladele et al., 2009).

It has been shown that B cells may not be the sole target for the virus. Macrophages may serve as host cells for IBDV (Khatri et al., 2005; Käufer and Weiss, 1976; Savova and Bozhkov, 1985; Palmquist et al., 2006). Macrophages, on the one hand, can phagocytize and clear pathogens by recognizing pathogen-associated molecular patterns (PAMPs) via their pattern-recognition receptors (PRRs) in innate immune system (Akira et al., 2006). On the other hand, macrophages secrete a variety of functional cytokines which can act as immunomodulatory, antiviral or proinflammatory factors, such as typeI interferon (IFN-α and IFN-β), IL-6, TNF-α, etc (Zhou, 2007). However, the role of macrophages in the pathogenesis of IBDV has not been extensively examined so far. It has been reported that gut-associated macrophages are hypothesized to be the initial transporters of IBDV from the digestive tract to the bursa and other peripheral organs (Käufer and Weiss, 1976). There are reports that infection with IBDV causes the production of proinflammatory mediators and cytokines by macrophages. The levels of induced proinflammatory mediators and cytokines reached the peak during active virus replication and correlated with extensive inflammatory response in both chicken bursa and spleen (Kim et al., 1998; Khatri et al., 2005; Palmquist et al., 2006). These macrophages were activated by viral infection and upregulated mRNA expression of IL-1β, IL-6, IL-18, and iNOS (Palmquist et al., 2006).

Kupffer cells (liver macrophages) represent one of the largest reservoirs of resident tissue macrophages, which are located in the liver sinusoids in close contact with endothelial cells. As for mammals, in addition to Kuffer cells, there are endothelial cells in the hepatic sinusoid. The Disse's space of liver harbors fat-storing cells and pit cells (Wisse et al., 1996). There are some distinctive characteristics of bile ductules and sinusoidal cells between mammals and chickens, although the ultrastructure of chicken liver is principally similar to that of rat or human liver. Investigation of ultrastructural chicken liver revealed that intercalated cells (extra-sinusoidal macrophages of chicken liver), a unique kind of chicken hepatic parenchyma cells, mostly present in interhepatocytic or Disse's spaces (Ohata et al., 1982; Ohata and Ito, 1986; Ghoddusi and Kelly, 2004).

So far, little is known about the pathological damage of virus replication to chicken livers and the susceptibility of Kupffer cells to virus during the acute phase of the IBDV infection. Thus, in the present study we examined the impacts of IBDV infection on chicken liver tissues and, in particular, on Kupffer cells by immunohistochemisrty and transmission electron microscopy (TEM).

2. Materials and methods

2.1. Animals, viruses and rabbit anti-IBDV serum

The specific-pathogen-free (SPF) male white Leghorn chickens were purchased from Merial Vital Laboratory Animal Technology Co., Ltd. (Beijing, China) and reared in CC. JH-1 type positive pressure isolation units which were purchased from Jinhang Purifying Air Conditioning Equipment Co., Ltd. (Tianjin, China) under the supervision of the Animal Facility Management of China Agricultural University. Chickens for different experimental groups were housed in the separate isolation units. Water and feed were provided ad libitum during the entire experimental period. Virulent strain of IBDV (BC6/85, CVCC AV7) was obtained from China Institute of Veterinary Drug Control (Beijing, China). At 3 weeks of age, chickens were inoculated with 1000 50% egg infective doses (EID50) of IBDV strain BC6/85 or phosphate-buffered saline (PBS) by eye drop. Rabbit anti-IBDV serum was produced as previously described (Tanimura et al., 1995; Oladele et al., 2009). And the titer of rabbit antiserum was 1: 16 by agar gel precipitation (AGP) assay. Both IBDV (BC6/85) antigen and chicken anti-IBDV serum as a positive control in AGP assay were obtained from China Institute of Veterinary Drug Control (Beijing, China).

2.2. Experimental design

A total of seventy 3-week-old SPF chickens were randomly distributed into two groups (the IBDV-exposed group and the control group) of 35 birds each, and were separately weighed before their sacrifice. IBDV-exposed birds were inoculated with IBDV strain BC6/85 and were euthanized (5 birds once) at 12, 24 h post infection (h.p.i.) and 2, 3, 4, 5, 6 days post infection (d.p.i.), and control birds were inoculated with PBS and were euthanized (5 birds once) at the same intervals. Of these chickens, the organs of bursa of Fabricius from both infected and uninfected chickens were sampled and weighed. The bursal weight index was calculated by dividing the value of bursal weight (g) by the value of chicken body weight (kg).

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