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Research paper

Reduced mucosal injury of SPF chickens by mast cell stabilization after infection with very virulent infectious bursal disease virus

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ABSTRACT

Recent studies here have demonstrated that increased mast cell populations and tryptase activity contribute to lesion formation in regions of immune organs in special-pathogen-free chickens after infection with very virulent infectious bursal disease virus (vvIBDV). Mast cells and their mediators have been implicated in acute inflammatory injury after vvIBDV infection, but their precise role in this process remains elusive. In this study, the role of mast cells in the vvIBDV infection process was examined using ketotifen, a mast cell membrane stabilizer. On days 1, 2, and 3 postinfection, the bursa of Fabricius (BFs) were collected to quantify mast cells, tryptase and histamine contents by cytochemistry, immunohistochemistry and fluorospectrophotometry analyses, respectively. The results showed that the mast cell populations, tryptase expression, and histamine released increased significantly in the BFs ($p < 0.01$) of infected birds compared to controls, and acute inflammatory responses were observed in the former. In contrast, in infected chickens pretreated with ketotifen, mast cells, tryptase, and histamine were markedly decreased ($p < 0.01$) and probably as a result, the BFs remitted significantly. The overall results suggest that mast cells are positively involved in BF injury induced by vvIBDV infection. Inhibition of mast cell degranulation and concurrent mediator release may represent a novel strategy to modulate this process. This study, thus, advances the understanding of the acute inflammatory injury mechanisms triggered by vvIBDV infection and the contribution of mast cell activity in this process.

1. Introduction

Infectious bursal disease viruses (IBDV), of the family *Birnaviridae*, are significant, widespread pathogens in the poultry industry. IBDV destroys B lymphocytes in the bursa of young chickens and causes infectious bursal disease (IBD), an acute, highly contagious immuno-suppressive disease among young chickens (Becht and Müller, 1991; van den Berg, 2000). First isolated in the town of Gumboro, DE, USA in 1957 (Cosgrove, 1962), IBDV has caused enormous worldwide economic loss in the poultry industry. Very virulent (vv) IBDV strains emerged in Europe in the late 1980s and have caused about 70% mortality in chickens in several European and Asian countries (Nunoya et al., 1992; van den Berg et al., 1991; Zhou et al., 1982). In spite of industrial vaccination

Abbreviations: IBD, infectious bursal disease; IBDV, infectious bursal disease virus; vvIBDV, very virulent infectious bursal disease virus.

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programs, IBD continues to be a serious economic problem largely due to the spread and subsequent outbreak of vvIBDV (Saif, 1998; van den Berg, 2000), causing a higher incidence of acute disease and higher mortality (Nunoya et al., 1992; Snyder et al., 1992; van den Berg, 2000). In addition the virus-induced immune suppression results in secondary infections, growth retardation, and condemned carcasses at slaughter. It is generally accepted that the antigenicity and pathogenic signs of vvIBDV are similar to classical serotype I IBDV, but antibodies, including maternal antibodies, induced by attenuated serotype I vaccine do not provide satisfactory protection against vvIBDV (van den Berg, 2000).

Currently, the depletion of B-lymphocytes in the bursa is known to be a crucial element in the early phase of vvIBDV infection, but it is not clear whether this effect is due to apoptosis or necrosis (Clouda et al., 1992; Jungmann et al., 2001; Käufer and Weiss, 1980; Müller et al., 2003). Although severe inflammation damage is a well documented consequence of IBDV infection, the exact mechanism of lymphocyte depletion is still not well characterized. Recently, the role of some immune cells, such as macrophages and mast cell, has attracted much attention from veterinary researchers (Khatri et al., 2005; Wang et al., 2008). Results from Khatri have demonstrated that IBDV can target macrophages and induce the release of mediators, ultimately resulting in inflammatory lesions in infected animals (Khatri et al., 2005). A previous study here revealed that mast cell populations and tryptase activity increased significantly in vvIBDV-infected birds (Wang et al., 2008). However, the exact process involved in mast cell switching to cause inflammatory injury of the bursa of Fabricius (BFs) in chickens infected with vvIBDV is still under debate.

Mast cells, the key "effector cells" in inflammation, are resident in tissues throughout the body and particularly in tissues associated with structures such as skin, blood vessels and nerves, and in the tissues that interface with the external environment (Galli et al., 1999; Metcalfe et al., 1997). The critical role of mast cells in the immune response and in inflammation and physiological processes became clear after evidence indicated that mast cells are responsible for the production of many inflammatory molecules (Gordon and Galli, 1990; Metcalfe et al., 1997). As long-lived cells, mast cells play various roles in viral invasion, possibly contributing to the initiation of inflammatory responses (Kaliner, 1987; Kobayashi et al., 2000), or act as reservoirs, aiding viruses, such as macrophage-tropic HIV-1 (Bannert et al., 2001), Dengue virus (King et al., 2000), cytomegalovirus (Gibbons et al., 1990) and adenovirus (King et al., 2002), by eluding immunosurveillance and hastening the inflammatory response. A preliminary study conducted by Sun et al. demonstrated that increased mast cell density in specific-pathogen-free (SPF) chickens after infection with New Castle Disease virus (NDV) was consistent with the infiltration of inflammatory cells and histopathological changes (Sun et al., 2008). Recently, it was found that mast cell populations were significantly increased in vvIBDV-infected SPF chickens. More interesting, mast cell populations and tryptase activity were markedly increased in the BFs, also showing the positive relevance of vvIBDV distribution to vvIBDV antigen and tryptase content (Wang et al., 2008). These findings was found that mast cells degranulation might be a possible mechanism by which pathogens induced an inflammatory response, and that it is important to identify the exact mechanisms by which they may provide protection. Some studies have demonstrated that mast cell stabilization prevents histological damage of gastric mucosa induced by agents such as ethanol (Kalia et al., 2000; Kalia et al., 1997; Oates and Hakkinen, 1988), but it is not known whether they also prevent damage induced by vvIBDV in SPF chickens. In summary, these microscopic observations of mast cells, taken together with histological and cytochemical staining results, demonstrate changes that are indicative of the degranulation of mast cells.

Tryptase and histamine are regarded as the principal products of activated mast cells (Fox et al., 1982; He and Walls, 1997; Metcalfe et al., 1997; Miadonna et al., 1994) and the release of these two factors from degranulated mast cells is the best known agent for a diversity of roles in many processes. Thus, the goal of the current study was to evaluate the effects of mast cell stabilization and its mediator antagonism on vvIBDV-induced acute inflammation using histochemical and histologic methods and to study the mechanisms by which mast cell stabilization may protect against inflammatory damage.

2. Materials and methods

2.1. Animals and experimental treatments

One hundred and five 30-day-old, healthy, SPF chickens (White Avian, Merial Co. Ltd., Beijing, China), negative to NDV and IBD virus, were divided randomly into three groups (45 birds each in the vvIBDV infected and ketotifen pretreated groups, 15 birds as the control), which were then separated in individual negative-pressure isolators. All chickens were provided feed and water *ad libitum*.

The vvIBDV SNJ93 strain was obtained from the China Institute of Veterinary Drug Control (Beijing, China) and had an estimated LD_{50} (eLD_{50}) of vvIBDV at 3.2×10^6/0.2 mL, and was diluted 1/100 in sterile saline solution. Chickens in the vvIBDV infected group were inoculated with 0.2 mL of the vvIBDV dilution by means of nasal and eye drops. In the ketotifen pretreated group, birds were pretreated with 1 mL ketotifen (Sigma, Beijing, 1 mg/kg body weight), dissolved in 0.9% saline and prepared fresh on the day of use, by a stainless steel orogastric tube approximately 1 h before infection. In the control group, chickens received equal volumes of saline by the same route. Animals were visually examined daily, euthanasia was performed by intravenous injection of sodium pentobarbital in a brachial vein, and necropsies performed immediately postmortem. This study was approved by the Institutional Animal Care and Use Committee of China Agricultural University.

2.2. Sampling

On days 1, 2, and 3 postinfection, 15 chickens from each test group and 5 control birds were randomly selected,