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Research paper

Differences in genetic background influence the induction of innate and acquired immune responses in chickens depending on the virulence of the infecting infectious bursal disease virus (IBDV) strain

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ABSTRACT

Previous studies and field observations have suggested that genetic background influences infectious bursal disease virus (IBDV) pathogenesis. However, the influence of the virulence of the infecting IBDV strain and the mechanisms underlying the differences in susceptibility are not known. In the present study IBDV pathogenesis was compared between specific-pathogen-free layer-type (LT) chickens, which are the most susceptible chicken for IBDV and have been used as the model for pathogenesis studies, and broiler-type (BT) chickens, which are known to be less susceptible to clinical infectious bursal disease (IBD). The innate and acquired immune responses were investigated after inoculation of an intermediate (i), virulent (v) or very virulent (vv) strain of IBDV. IBDV pathogenesis was comparable among genetic backgrounds after infection with iIBDV. After infection with vIBDV and vvIBDV, LT birds showed severe clinical disease and mortality, higher bursal lesion scores and IBDV-antigen load relative to BT birds. Circulating cytokine induction varied significantly in both timing and quantity between LT and BT birds and among virus strains ($P < 0.05$). Evaluation of different immune cell populations by flow-cytometric analysis in the bursa of Fabricius provided circumstantial evidence of a stronger local T cell response in BT birds vs. LT birds after infection with the virulent strain. On the other hand, LT birds showed a more significant increase in circulating macrophage-derived immune mediators such as total interferon (IFN) and serum nitrite than BT birds on days 2 and 3 post-vIBDV infection ($P < 0.05$). Stronger stimulation of innate immune reactions especially after vIBDV infection in the early phase may lead to faster and more severe lesion development accompanied by clinical disease and death in LT chickens relative to BT chickens. Interestingly, no significant differences were seen between genetic backgrounds in induction of the IBDV-specific humoral response: timing of IBDV-antibody induction and antibody levels were comparable between BT and LT birds. This study clearly demonstrates a significant influence of chickens' genetic background on disease outcome. The difference between backgrounds in IBDV susceptibility is further influenced by the virulence of the infecting virus strain.

Abbreviations: BrL, brown Leghorn; BF, bursa of Fabricius; BSA, bovine serum albumin; BT, broiler-type chicken; CEF, chicken embryo fibroblasts; CPE, cytopathic effect; ELD, egg lethal dose; FBS, fetal bovine serum; HE, hematoxylin and eosin; IBD, infectious bursal disease; IBDV, infectious bursal disease virus; i, intermediate; Ig, immunoglobulin; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; IM, Irwin Moulthroph; LT, layer-type chicken; MHC, major histocompatibility complex; NO, nitric oxide; OD, optical density; PBS, phosphate-buffered saline; pi, post-inoculation; SPF, specific-pathogen-free; VN, virus neutralization; VSV, vesicular stomatitis virus; v, virulent; vv, very virulent.

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1. Introduction

Infectious bursal disease virus (IBDV) causes an acute, highly contagious, immunosuppressive disease in chickens (Eterradossi and Saif, 2008). The causative agent belongs to the *Birnaviridae* family. Different pathotypes of IBDV have been classified in increasing order of virulence as mild, intermediate (i), classical virulent (v) and very virulent (vv). Classical virulent IBDV strains isolated from the United States in the early 1960s, such as the Irwin Moulthroph (IM) strain, induce hemorrhagic lesions accompanied by depletion of B cell follicles and mortality rates of 30–60% in layer-type chickens. In the mid 1990s, "very virulent" strains of IBDV, causing >70% mortality in chickens, emerged in several European and Asian countries. van den Berg (2000) suggested that vvIBDV causes clinical signs similar to those of the classical virulent strains with the same incubation time of 4 days but with an exacerbated acute phase.

Chickens are highly susceptible to IBDV between 3 and 6 weeks after hatching. Experiments in which bursectomized chickens survived IBDV infection demonstrated that the bursa of Fabricius (BF) is the main target organ for IBDV. The acute phase usually lasts about 1 week, and peak clinical signs and mortality are recorded between 3 and 4 days after IBDV infection. The most common mode of IBDV infection is via the oral route. The virus is taken up from the gut and transported to other tissues by phagocytic cells, most likely resident macrophages. IBDV infects and destroys actively dividing IgM-bearing B cells in the BF (Rodenberg et al., 1994). Although B cells are the principal targets for IBDV, recent data show that the virus also infects and replicates in macrophages. Infection with IBDV causes the production of proinflammatory mediators and cytokines in macrophages, which peaks during the early phase of active virus replication (Khatri et al., 2005; Palmquist et al., 2006). IBDV induces expression of the following cytokines and cytokine genes: interleukin (IL)-12, interferon (IFN)-γ, IL-1β, IL-6 and CXCLi2 in bursal cells (Eldaghayes et al., 2006; Rauw et al., 2007), and expression of IL-1β, IL-6, IL-18 and inducible nitric oxide synthase (iNOS) in spleen cells (Palmquist et al., 2006). Nitric oxide (NO), which is produced by activated macrophages, may promote cellular destruction of both virus-infected and virus-free cells (Yeh et al., 2002). T cells, which are not infected by IBDV, may modulate the pathogenesis by limiting viral replication in the BF during the early phase of the disease at 5 days pi, by promoting bursal tissue damage and delaying tissue recovery, possibly through the release of cytokines and their concomitant cytotoxic effects (Rautenschlein et al., 2002a). Almost all these basic studies on IBDV pathogenesis have been done in specific-pathogen-free (SPF) layer-type chickens. Not much is known about the genetic influence on immune cell reactions in other types of chickens, which may show different susceptibility to infectious bursal disease (IBD) compared to SPF-layer-type chickens. It is difficult to compare the influence of genetic background on IBD with different commercially available chicken lines due to the maternal antibody levels, which may vary between birds of different background and parent flock in titer and half life time (de Wit, 1998). Previous preliminary studies indicated that detectable maternal antibodies above the break-through titer of the infecting intermediate (i) IBDV strain may delay virus replication and the induction of lesions but not the replication rate and severity of lesions in comparison to birds with antibody levels below the break-through levels or without detectable antibodies (Block et al., 2007; Jung, 2006).

Some chicken lines with different major histocompatibility complex (MHC) haplotypes have been investigated following IBDV infection. Although no relation between MHC haplotype and resistance to IBDV has been observed to date, major differences have been found between different chicken lines. Bumstead et al. (1993) reported various mortality rates after infection of 11 inbred and partly inbred chicken lines with vvIBDV, being highest in a brown Leghorn line and lowest in some white Leghorn lines. Hassan et al. (2002) also reported major differences in mortality rates among six genetically different chicken lines. A recent study by Ruby et al. (2006) revealed differences in the transcript levels of some inflammatory- and immune cell-related genes during the acute phase of IBD between brown layer and white layer lines known to differ in IBD susceptibility. A notable MHC haplotype effect was observed on the specific antibody response against an inactivated IBDV as measured by ELISA (Juul-Madsen et al., 2006).

The goal of this study was to understand the immune mechanisms contributing to the development of clinical disease and mortality. Our objectives were to compare innate and acquired immune reactions in the acute phase of IBDV infection between commercial Ross-type broilers (BT), known to resist clinical IBD under experimental conditions, and highly susceptible SPF-layer-type chickens (LT), which have been used extensively in many IBDV pathogenesis studies. The immune responses were determined after infection of BT and LT chickens with IBDV strains of different virulence – iIBDV, vIBDV, and vvIBDV. The development of clinical disease, macroscopic and microscopic lesions, IBDV-antigen load in the BF, IBDV-antibody development, and the induction of circulating cytokines were determined during the acute phase of IBD. Serum samples were tested for serum nitrite and the following cytokines: IL-6, total IFN, IFN-γ, and IL-1β, which are upregulated after IBDV infection (Eldaghayes et al., 2006; Rauw et al., 2007).

2. Material and methods

2.1. Virus

The classical vIBDV strain IM was inoculated by the eyedrop route (Kim et al., 1999) at 10^4 egg lethal dose $(ELD)_{50}$/bird. The vvIBDV strain 89163/7.3 (provided by N. Eterradossi, AFSSA, Ploufragan, France) was also inoculated by the eyedrop route at a dose of 10^3 ELD_{50}/bird. These infectious doses for vIBDV and vvIBDV were chosen based on preliminary studies to reproduce the clinical disease in SPF LT chickens within 48 h pi. vIBDV and vvIBDV strains were propagated in 3-week old specific-pathogen-free (SPF) chickens (Rautenschlein et al., 2005). At 5 days after