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SCIENCE DIRECT
Veterinary Immunology and Immunopathology 106 (2005) 139–150
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Differences in the immunopathogenesis of infectious bursal disease virus (IBDV) following in ovo and post-hatch vaccination of chickens

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Received 21 September 2004; received in revised form 19 January 2005; accepted 1 February 2005

Abstract

Not much is known about IBDV-pathogenesis and immune mechanisms following in ovo vaccination. In this study, we compared the immunopathogenesis of an intermediate IBDV-vaccine in post-hatch- and in ovo-inoculated chickens. In ovo-vaccinated birds recovered significantly faster from lesions of the bursa of Fabricius than post-hatch vaccinated ($P < 0.05$). A significant accumulation of intrabursal CD8+ T cells was observed in post-hatch but not in in ovo-vaccinated chickens ($P < 0.05$). The innate immunity was comparable between in ovo- and post-hatch-vaccinated groups as indicated by comparable intrabursal macrophage accumulation and intrabursal IBDV-clearance. Overall, our observations indicate that IBDV in ovo vaccination may be advantageous over post-hatch. In ovo-vaccinated birds recover faster from bursa lesions and exhibit similar protection against challenge in comparison to post-hatch vaccinated.

Keywords: In ovo; Post-hatch; Infectious bursal disease; Chicken; Immunopathogenesis; Vaccination

1. Introduction

Infectious bursal disease (IBD) is an immunosuppressive disease in chickens (Lukert and Saif, 2003). Despite vigorous vaccination strategies, IBD is still an economically important disease in commercial poultry. The most common way of infectious bursal disease virus (IBDV) vaccine delivery in the field is by drinking water post-hatch, but also in ovo vaccination has been shown to induce protective immunity (Gagic et al., 1999; Coletti et al., 2001; Corley et al., 2001; Giambrone et al., 2001; Sharma

Abbreviations: B/BW, bursa to body weight ratio; CT, caecal tonsil; ELD, egg lethal dose; IBD, infectious bursal disease; IBDV, infectious bursal disease virus; PV, post vaccination; rpm, rounds per minute; S/BW, spleen to body weight ratio; SPF, specific pathogen-free; TCID, tissue culture infectious dose

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doi:10.1016/j.vetimm.2005.02.011

et al., 2002). Not much is known about the immunopathogenesis and the mechanisms of immunity following in ovo vaccination in chicken embryos lacking a fully mature immune system (Ahmad and Sharma, 1993; Reddy et al., 1996; Karaca et al., 1998; Gagic et al., 1999; Rautenschlein et al., 1999). So far, studies comparing the efficacy of in ovo versus post-hatch vaccination against IBD have focused on the induction of systemic humoral immunity and vaccine-induced lesions of the bursa of Fabricius (Gagic et al., 1999; St. Hill and Sharma, 1999; Coletti et al., 2001; Corley et al., 2001; Giambrone et al., 2001; Sharma et al., 2002), but recent studies indicated that humoral systemic immunity might not be the only mode of protection against IBDV challenge (Rautenschlein et al., 2002a). The induction of local immunity may also play an important role for protection because IBDV enters the circulation through gut-associated tissue before it is distributed to other organs (Müller et al., 1979). The induction of local gut-associated immunity and the impact of in ovo versus post-hatch vaccination on the non-specific and specific cell-mediated immunity is not known.

To optimise vaccination strategies in the field, and for the evaluation of IBDV vaccines it is essential to gain more knowledge about the modes of immune stimulation by IBDV-vaccines. Furthermore, possible differences in vaccine virus pathogenesis should be considered depending on the route and age of vaccine delivery. In this study, the immunopathogenesis of an intermediate IBDV-vaccine in post-hatch- and in ovo-vaccinated chickens was compared. The induction of bursa lesions and recovery, distribution of intrabursal T-cell populations and macrophage-like cells, IBDV-antigen distribution and clearance, the induction of humoral gut-associated and systemic immunity, and protection against challenge with classic virulent IBDV were determined.

2. Material and methods

2.1. Chickens

Specific pathogen-free (SPF) chickens, hatched from VALO® eggs, or embryonated eggs (VALO®) were obtained from Lohmann Tierzucht (Cuxhaven, Germany). The birds belonged to the genetic background white Leghorn, line Lohmann LSL-LITE. Chickens were hatched and reared in pressurised isolation units (Montaim Van Stratum, Kronsberg, Netherlands) following the guidelines of the Animal Care Committee for the duration of the study. The birds were given food and water ad libitum. Birds from different experimental groups were housed in separate isolation units.

2.2. Virus

An intermediate vaccine strain of IBDV (Bursine 2; Sharma et al., 2000) was propagated and titrated in chicken embryo fibroblast cultures (Kim et al., 2000). Birds were inoculated with 100 μl of 10^3 (Experiment 1), or 10^4 (Experiment 2 and 3) tissue culture infectious $dose_{50}$ ($TCID_{50}$) of IBDV/bird per eye drop (Experiment 1) or orally (Experiment 2 and 3) at 14 days post-hatch, or in ovo at embryonation day 18. The IBDV strain IM was used as a challenge virus at a dose of 10^3 egg-lethal dose $(ELD)_{50}$/bird inoculated by eye drop route (Kim et al., 1999). IBDV-IM was propagated in 3-week-old SPF chickens. At 5 days post-IBDV inoculation, bursae from infected birds were harvested, homogenised, and titrated in embryonated chicken eggs as previously published (Kim et al., 1999; Tanimura and Sharma, 1997).

2.3. H&E staining

For the detection of histopathological lesions, the bursa of Fabricius, spleen, and caecal tonsils were collected, fixed in 10% phosphate-buffered formalin and stained with hematoxylin and eosin (H&E). Lesions were observed microscopically. Bursa lesion scores were determined and compared between groups (Kim et al., 1999; Sharma et al., 1989). The scoring was as follows: 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of follicles showing cellular depletion.

2.4. Immunohistochemical detection of different immune cell populations and IBDV-antigen

For the detection of different immune cell populations, the following monoclonal antibodies were used: CVI-ChNL-74.2, which detects macrophages (Jeurissen et al., 1992); CT8 (Chan et al., 1988), specific for