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# Host responses in the bursa of Fabricius of chickens infected with virulent Marek's disease virus

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ABSTRACT

The bursa of Fabricius serves as an important tissue in the process of Marek's disease virus (MDV) pathogenesis, since B cells of the bursa harbor the cytolytic phase of MDV replication cycle. In the present study, host responses associated with MDV infection in the bursa of Fabricius of chickens were investigated. The expression of MDV phosphoprotein (pp)38 antigen, MDV glycoprotein (gB) and MDV viral interleukin (vIL)-8 transcripts was at the highest at 4 days post-infection (d.p.i.) and then showed a declining trend. On the contrary, the expression of meq (MDV EcoRI Q) gene as well as the viral genome load increased gradually until day 14 post-infection. The changes in viral parameters were associated with significantly higher infiltration of macrophages and T cell subsets, particularly CD4+ T cells into the bursa of Fabricius. Of the genes examined, the expression of interferon (IFN)-α, IFN-γ genes and inducible nitric oxide synthase (iNOS) was significantly up-regulated in response to MDV infection in the bursa of Fabricius. The results suggest a role for these cells and cytokines in MDV-induced responses in the bursa of Fabricius.



## Introduction

Marek's disease virus (MDV) is an alphaherpesvirus, which belongs to the genus *Mardivirus* (Davison et al., 2002). MDV infection in chickens leads to the formation of T cell tumors in various body tissues, neurological manifestations as well as immune suppression (Calnek, 2001; Payne, 2004). Feather dander and poultry house dust serve as the source for inhalation of infectious MDV and subsequent establishment of natural infection (Beasley et al., 1970; Calnek et al., 1970). Initial respiratory infection is followed by the cytolytic phase that extends from 3–6 days post-infection (d.p.i.) and occurs in lymphoid organs such as spleen, the bursa of Fabricius and thymus (Calnek, 2001). MDV established in the respiratory system is carried to other organs by infected macrophages (Barrow et al., 2003). In lymphoid organs, mainly B cells are infected initially by MDV (Shek et al., 1983; Calnek et al., 1984; Baigent et al., 1998). The importance of the bursa of Fabricius for the successful completion of MDV replication cycle has been shown (Schat et al., 1981). The absence of this phase, leads to lower viral replication resulting in lower viraemia and decreased incidence of tumor formation (Schat et al., 1981). Following a burst of productive/restrictive infection in B cells that is associated with high transcriptional activity of MDV phosphoprotein (pp)38 antigen (Baigent et al., 1998; Burgess et al., 2001; Burgess and Davison, 2002), a switch to latent infection in T cells occurs approximately 7 d.p.i. The switching of infection may be influenced by a protein encoded by MDV, viral interleukin (vIL)-8 that acts as a chemoattractant for chicken T cells (Liu et al., 1999; Parcells et al., 2001) and allows the infiltration of T cells to the vicinity of MDV-infected B cells. Alternatively, MDV-infected cells can up-regulate major histocompatibility complex (MHC) class II molecules on MDV-infected cells (Niikura et al., 2007) which may facilitate presentation of MDV antigens (Malnati et al., 1992) and initiation of host response, allowing T cell infiltration into the site of virus replication. MDV infection in T cells becomes latent probably due to host responses elicited by the virus (Buscaglia et al., 1988; Volpini et al., 1996). The latent phase is followed by transformation of T cells and tumor formation in Marek's disease (MD) susceptible chickens (Calnek, 2001).

Host responses elicited against MDV infection have been studied in spleen, which serves as a major secondary lymphoid organ in the chicken and harbors almost all the stages of the MDV replication cycle (Xing and Schat, 2000a; Kaiser et al., 2003; Jarosinski et al., 2005; Sarson et al., 2006). Recently, studies have been focused on elucidating host responses at the site of virus shedding (Abdul-Careem et al., 2008), in the central nervous system (Gimeno et al., 2001; Jarosinski et al., 2005; Abdul-Careem et al., 2006b) and also in blood (Quere et al., 2005), since all these body compartments are engaged in different phases of the MDV replication cycle. The cytolytic phase is an important phase of the MDV replication cycle that mainly involves the bursa of Fabricius (Schat et al., 1981) and the consequence of this phase is usually immune suppression leading to increased susceptibility to other secondary infections (Islam et al.,

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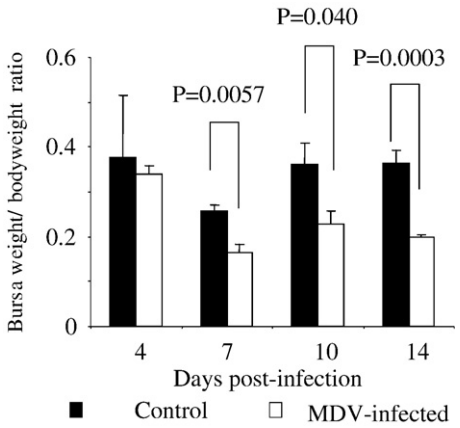


**Fig. 1.** Bursa/bodyweight ratios of chickens infected with RB1B strain of MDV and uninfected controls. The groups were as follows: MDV-infected=chickens that were infected with MDV and sampled at 4, 7, 10 and 14 d.p.i. and Control=age-matched chickens that were not infected. There were four chickens in each group at each time point. Differences between groups were assessed by student t test and comparisons were considered significant at $P \leq 0.05$.

2002). Although, cell-mediated immune responses as characterized by the infiltration of T cells and macrophages (Gimeno et al., 2001; Abdul-Careem et al., 2008) and expression of cytokine genes (Xing and Schat, 2000a; Kaiser et al., 2003; Jarosinski et al., 2005; Quere et al., 2005; Abdul-Careem et al., 2006b, 2008) have been investigated in various tissues, there is no information available on host responses in the bursa of Fabricius following MDV infection. This is an important lymphoid organ, which perform both primary as well as secondary immune functions.

Host responses characterized by cell infiltration and expression of cytokine genes have been studied in the bursa of Fabricius in response to infectious bursal disease virus (IBDV) infection in chickens (Tanimura and Sharma, 1997; Kim et al., 1999, 2000; Rautenschlein et al., 2002a; Khatri et al., 2005; Eldaghayes et al., 2006; Palmquist et al., 2006). These responses, especially T cell responses, have been shown to be effective in controlling IBDV replication (Kim et al., 2000; Rautenschlein et al., 2002a,b). In terms of cytokine responses, high transcriptional activities of interferon (IFN)-γ (Kim et al., 2000; Rautenschlein et al., 2003; Eldaghayes et al., 2006), IFN-β (Eldaghayes et al., 2006) and proinflammatory cytokines such as IL-18 (Khatri et al., 2005; Palmquist et al., 2006) and IL-6 (Kim et al., 2000; Eldaghayes et al., 2006; Khatri et al., 2005; Palmquist et al., 2006) have been shown in the bursa of Fabricius in response to IBDV infection.

Given the paucity of information on the mechanisms of host response to MDV interaction in the bursa of Fabricius, which carries an important phase of the MDV life cycle, the objective of the present study was to investigate the cellular and cytokine responses in this lymphoid organ subsequent to MDV infection.

## Results

### *Weight of the bursa of Fabricius of MDV-infected and -uninfected chickens*

Bursa weight as a percentage of bodyweight of MDV-infected and uninfected control chickens are illustrated in Fig. 1. The bursa/

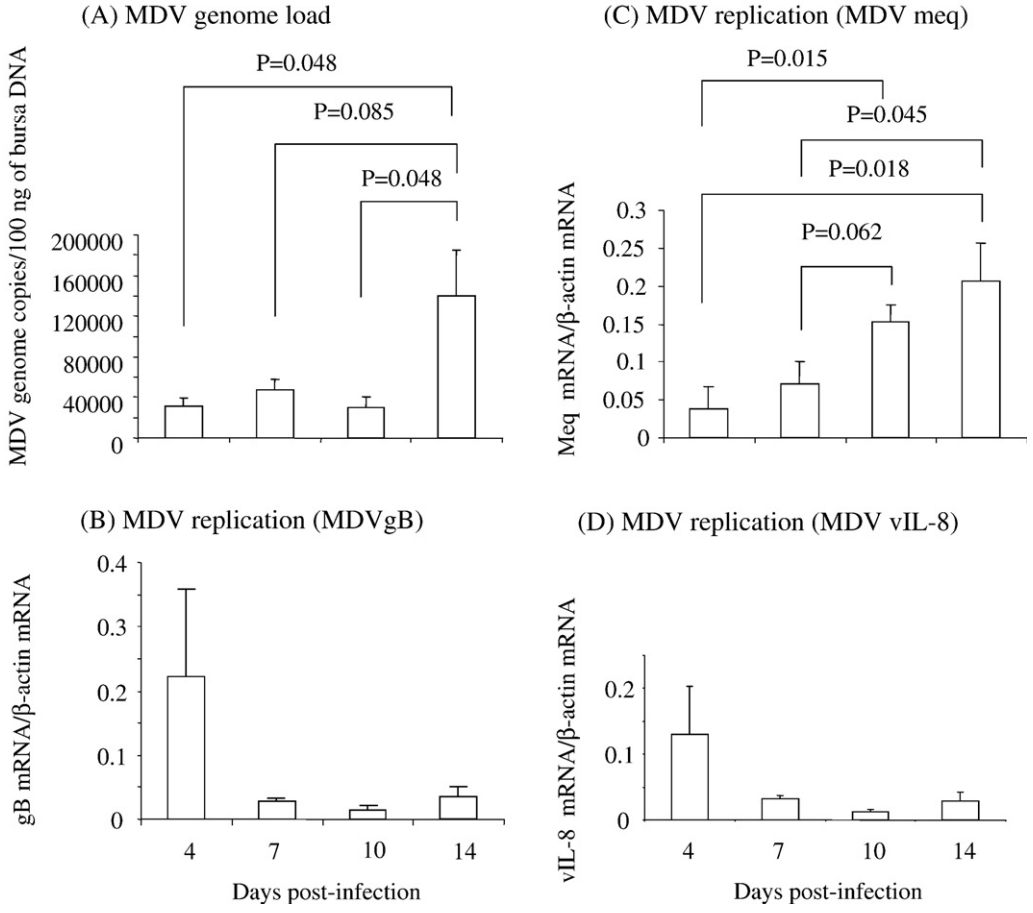


**Fig. 2.** MDV genome load and MDV transcripts in bursa of Fabricius of chickens infected with RB1B strain of MDV. Chickens were infected with MDV and sampled at 4, 7, 10 and 14 d.p.i. There were five MDV-infected chickens at each time point. Mean MDV genome load (A), gB mRNA (B), meq mRNA (C) and vIL-8 mRNA (D) expression relative to β-actin mRNA expression are presented and the error bars represent standard error of the mean.