# Expression profiles of genes within a subregion of chicken major histocompatibility complex B in spleen after Marek's disease virus infection

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**ABSTRACT** Major histocompatibility complex has previously been shown to influence the resistance of chicken to Marek's disease virus (MDV). However, little is known about expression of other genes in the MHC-I and II pathway after MDV infection. This study aimed at investigating 8 immune-related genes in the MHC core region that affects host responses to MDV. Spleens of infected and age-matched uninfected chickens were removed at 4, 7, 14, 21, and 28 d postinfection for gene expression detection using real-time PCR. Different expression patterns of MHC-I and II pathway genes were observed in the spleen. In the MHC-I pathway, the expression of transporter of antigen protein 1 (TAP1), transporter of antigen protein 2 (TAP2), and transporter of antigen protein-binding protein (TAPBP) genes was significantly increased in the spleen of MDV-infected than that of uninfected chickens. It indicated that host antivirus responses were generated to enhance antigen presentation. However, MHC-II pathway genes showed contrary trends. Classical MHC-II β chain major gene (BLB2) and nonclassical class II genes [DM α chain gene (DMA), DM β chain gene-1 (DMB1), and DM β chain gene-2 (DMB2)] had consistent lower transcripts in spleens of MDV-infected than that of uninfected chickens, which reflected that MDV interfered with multiple components of the MHC-II pathway. Overall, expression of most genes in the MHC core region was altered; moreover, the genes in endogenous and exogenous antigen presentation pathways had different expression patterns in the spleen after MDV infection.

**Key words:** chicken spleen, gene expression, Marek's disease, major histocompatibility complex

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## INTRODUCTION

Marek's disease virus (**MDV**) is a highly cell-associated, lymphotropic alphaherpesvirus that causes Marek's disease (**MD**) in chicken (Calnek and Witter, 1997). Like other herpesviruses, virulent strains of serotype 1 MDV infect cells both lytically and latently. An early cytolytic infection lasts from 3 to 6 d postinfection (**p.i.**) and targets primarily on B lymphocytes with temporary profound immunosuppression and T-cell activation (Calnek, 2001). After about 7 d p.i., the virus enters latency. Infected lymphocytes, mostly active T lymphocytes, migrate through the bloodstream to various tissues including the feather follicle epithelium, which is the sole source of the enveloped fully infectious virus (Calnek et al., 1970; Johnson et al., 1975; Calnek, 2001). After 21 d p.i., infected lymphocytes become neoplastically transformed and, in susceptible chickens, proliferate to form gross lymphomas (Calnek, 2001). Although vaccines for MDV have been used for over 30 yr, MDV virulence presents an increasing trend on the pressure of vaccines. Thus, MD continues to be a serious threat to the health of poultry. Recently, there has been a focusing on MD resistance of chickens and involved are several genetic loci including the MHC (Dalgaard et al., 2003, 2005; Shaw et al., 2007; Shiina et al., 2007; O'Neill et al., 2009).

The MHC plays pivotal roles in host immune surveillance and is of great importance in disease resistance. In general, MHC participates in the development of both humoral and cell-mediated immune responses. There are 2 different approaches, the MHC-I and II pathway, to present intracellular and extracellular antigens, respectively. Briefly, endogenous pathogens are degraded into small fragments by proteasomes and then transported into the endoplasmic reticulum (**ER**) by 2 transporters of antigen proteins (**TAP**; TAP1 and TAP2) and 4


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TAP-binding proteins (**TAPBP**) in human (Deverson et al., 1990; Spies et al., 1990; Trowsdale et al., 1990; Ortmann et al., 1997). This arrangement allows the trimer of class I, β2 microglobulin and peptide to go to the cell surface and present pathogens to CD8+ T cells (Pamer and Cresswell, 1998). Exogenous antigens are presented to CD4+ T cells through a MHC-II molecule assisted by DM protein, which is encoded by DM α chain gene (**DMA**), DM β chain gene-1 (**DMB1**), and DM β chain gene-2 (**DMB2**). In brief, DM complex accumulates in the endosomal compartments (Sanderson et al., 1994) and facilitates the removal of the class II-associated invariant chain peptide (Denzin and Cresswell, 1995; Sloan et al., 1995), allowing exogenous peptide to bind to the peptide-binding region of the MHC-II molecule (Ghosh et al., 1995), and finally presents antigens to effector cells.

Therefore, it is not surprising that host regulates the expression of MHC as an immune defense at RNA or posttranscriptional levels during the course of infection. Simultaneously, many herpesviruses also develop strategies to interfere with expression of both MHC-I and MHC-II to counter host defenses (Miller and Sedmak, 1999; Vossen et al., 2002; Hegde et al., 2003; Ambagala et al., 2005). For example, herpes simplex virus interferes with antigen loading in the ER through a specific interaction between infected cell peptide 47 and TAP to downregulate MHC-I protein on the cell surface (Früh et al., 1995; Hill et al., 1995). Varicella zoster virus is known to downregulate MHC-I at the post-ER level through inhibiting TAP-mediated MHC complex (Abendroth and Arvin, 2001).

With respect to MDV, it can upregulate the expression of the MHC-I gene after MDV infection (Morgan et al., 2001; Abdul-Careem et al., 2008; Heidari et al., 2008). However, in most cases, MHC-I complex surface expression, especially α-chain, is showed to be downregulated after MDV infection (Hunt et al., 2001; Levy et al., 2003). Although the mechanism and viral protein(s) involved have yet to be elucidated, inhibition of MHC-I protein transporting to the cell surface is most likely the affected pathway (Hunt et al., 2001).

As for the MHC-II gene, its expression can also be downregulated by herpesviruses (Hegde et al., 2003). For instance, varicella zoster virus, human cytomegalovirus, mouse cytomegalovirus, and herpes simplex virus 1 suppress interferon (**IFN**)-induced MHC-II upregulation at the transcriptional level by disrupting the classic IFN pathway (Heise et al., 1998; Le Roy et al., 1999; Miller and Sedmak, 1999; Abendroth et al., 2000; Abendroth and Arvin, 2001; Yokota et al., 2001). Regarding MDV, however, its effects on MHC-II gene expression seem to be variable. It has been suggested that MDV directly upregulates the expression of MHC-II invariant chain (Ii chain) in chicken embryo fibroblasts (Niikura et al., 2007) and the central nervous system of infected chickens (Gimeno et al., 2001). But, Heidari et al. (2008) reported that expression of the MHC-II gene decreases in spleens of MDV-infected chickens. Expression of the MHC-II β chain gene is significantly downregulated at multiple time points (Sarson et al., 2008). To this contradictory observation, Thanthrige-Don et al. (2010) suggested that the influence of MDV on the MHC-II seems to vary depending on the experimental model.

Previous studies mentioned above mainly focused on MHC-I and II molecules and little is known about expression of other immune-related genes after MDV infection. In the present study, we endeavored to analyze other genes besides MHC-I and II in endogenous and exogenous pathways to profile their change throughout all stages of viral pathogenesis including cytolytic, latent, and transformation phases. Genes of emphases included MHC-I, TAP1, TAP2, TAPBP, classical MHC-II β chain major gene (**BLB2**), DMA, DMB1, and DMB2, which were located in the B-F/B-L region of chicken MHC-B on GGA 16. The tissue involved was the spleen, which plays a key role in the immune system.

## MATERIALS AND METHODS

### Infection Virus Strain and Experimental Birds

Chickens from a White Leghorn specific-pathogen-free line (BWEL) were infected with the MDV strain GA (passage 9) from American Type Culture Collection (Lian et al., 2010). Briefly, 40 one-day-old chicks were randomly divided into 2 groups. Twenty chickens were infected intraperitoneally with 2,000 pfu of the GA strain of virulent MDV. The rest were injected with the same dosage of diluent as the uninfected controls (0.2 mL). The 2 groups were housed in separate cages in the same filtered-air, positive-pressure isolation room. On 4, 7, 14, 21, and 28 d p.i., whole spleens from 4 infected chickens and 4 controls were removed and stored in RNA fixer (BioTeke Co. Ltd, Beijing, China). All of the samples were preserved at 4°C overnight and transferred to −80°C until analysis.

### RNA Extraction and cDNA Synthesis

Total RNA was isolated from spleens by RNAsimple total RNA Kit (Tiangen Biotech Co. Ltd., Beijing, China; Wu et al., 2008) and dissolved in RNase-free water. Reverse transcription was performed using 1 μg of total RNA in a total volume of 25 μL, using 10 m*M* oligo T primer and murine leukemia virus reverse transcriptase (Promega Biotech Co. Ltd, Beijing, China) according to the manufacturer's instructions.

### Primers

The primers specific for β-actin, TAP1, TAP2, TAPBP, BLB2, DMA, DMB1, and DMB2 genes were designed across exon and intron boundaries after alignment of the relevant nucleotide sequences in the Gen-

Bank database (accession no. X00182 and AB268588, respectively) using the Primer Express software (Version 3.0, Applied Biosystems, Foster City, CA). The primer for MHC-I followed that by Abdul-Careem et al. (2008). The primer sequences are listed in Table 1. The primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China).

### Preparation of Constructs as Standards and Real-Time PCR

Real-time PCR of gene expression was conducted using standard curves. For construction of standard curves of target and reference genes, PCR products of the reference gene (β-actin) and target genes were cloned into pMD 18-T Vector (Takara Bio Inc., Otsu, Japan) and 10-fold serial dilutions ($10^{-1}$ to $10^{-8}$) of the relevant plasmid DNA preparations were made and assayed in duplicate.

Each real-time PCR was run along with a dilution series of the standard that served as the calibrator. A no-template control was included with each run. All of the reactions were run in duplicate using ABI 7300 system (Applied Biosystems). The PCR were performed in a final volume of 15 μL with 1 μL of cDNA, 100 n*M* of each gene-specific primer, and 1× PCR mix (Power SYBR Green PCR Master Mix, Applied Biosystems). The optimum thermal cycling parameters included 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, 60°C for 30 s, and 95°C for 15 s.

### Data Analysis

The expression of MHC-related genes was calculated relative to the expression of the β-actin gene and expressed as ratios. The mean of the replicates from each of the 4 individual birds in infected and control groups per time point was subjected to Student's *t*-test using the SAS System Release 8.0 (SAS Institute Inc., Cary, NC) to identify treatment differences on each time point. Comparisons were considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$.

## RESULTS AND DISCUSSION

### Generation of Standard Curves for Gene Expression

Standard curves for relative quantification of MHC-I, TAP1, TAP2, TAPBP, BLB2, DMA, DMB1, and DMB2 genes were generated. The values recorded for slope of the curve were −3.250, −3.113, −3.448, −3.247, −3.365, −3.343, 3.191, and −3.390 and that yielded PCR efficiency of 1.03, 1.09, 0.95, 1.03, 0.98, 0.99, 1.06, and 0.97, respectively. The regression coefficients recorded for each were 0.997, 0.999, 0.998, 0.998, 0.994, 0.999, 1.000, and 1.000, respectively.

### Expression of Important Members in Endogenous Pathway

We detected expression of MHC-I, TAP1, TAP2, and TAPBP genes of the MHC-I pathway in spleens of MDV-infected and uninfected chickens. Although the difference was not significant at any age tested ($P > 0.05$), the expression of the MHC-I gene (Figure 1A) showed an increasing trend for higher values in spleens of MDV-infected than control chickens. This increasing trend in MDV-infected chickens is consistent with previous studies. For example, Morgan et al. (2001) have reported transcripts of MHC-I and β2 microglobulin increase in chicken embryo fibroblasts at 2 and 4 d p.i.

**Table 1.** Primers used in the real-time PCR analysis

| Gene[1] | Dir+ection | Sequence | Product (bp) |
|---|---|---|---|
| β-actin | Forward | 5′-GAGAAATTGTGCGTGACATCA-3′ | 152 |
| | Reverse | 5′-CCTGAACCTCTCATTGCCA-3′ | |
| MHC-I[2] | Forward | 5′-ACAAGTACCAGTGCCGCGTG-3′ | 197 |
| | Reverse | 5′-CGCGATGTTGTAGCCCTTCC-3′ | |
| TAP1 | Forward | 5′-TCGTCACCTTCCTCCTCTACCA-3′ | 123 |
| | Reverse | 5′-CCCGGTCCAGGAACTCAAA-3′ | |
| TAP2 | Forward | 5′-CGTCCCACCGTCCTTATCCT-3′ | 138 |
| | Reverse | 5′-CTTCTCCAGCATCCGTGGTT-3′ | |
| TAPBP | Forward | 5′-AGGACAAAGGCCACCAAGAA-3′ | 133 |
| | Reverse | 5′-ACGTCTACAGCTGCGTTGTCA-3′ | |
| BLB2 | Forward | 5′-CCCTCGGCGTTCTTCTTCTAC-3′ | 128 |
| | Reverse | 5′-CCCACGTCGCTGTCGAA-3′ | |
| DMA | Forward | 5′-CGTCACCCACCTCACCTACA-3′ | 145 |
| | Reverse | 5′-CACCCAGTAAGCCACCACAGA-3′ | |
| DMB1 | Forward | 5′-CGACACCGTCCACCTCATCT-3′ | 116 |
| | Reverse | 5′-CGTTGGGCAGCAGTTTGG-3′ | |
| DMB2 | Forward | 5′-TGCCTTCATGGTGCATGTG-3′ | 141 |
| | Reverse | 5′-CAGTCGCAGGCGTTGAAGA-3′ | |

[1]TAP1 = transporter of antigen protein 1; TAP2 = transporter of antigen protein 2; TAPBP = transporter of antigen protein-binding protein; BLB2 = MHC-II β chain major gene; DMA = DM α chain gene; DMB1 = DM β chain gene-1; DMB2 = DM β chain gene-2.

[2]Primers followed that by Abdul-Careem et al. (2008).

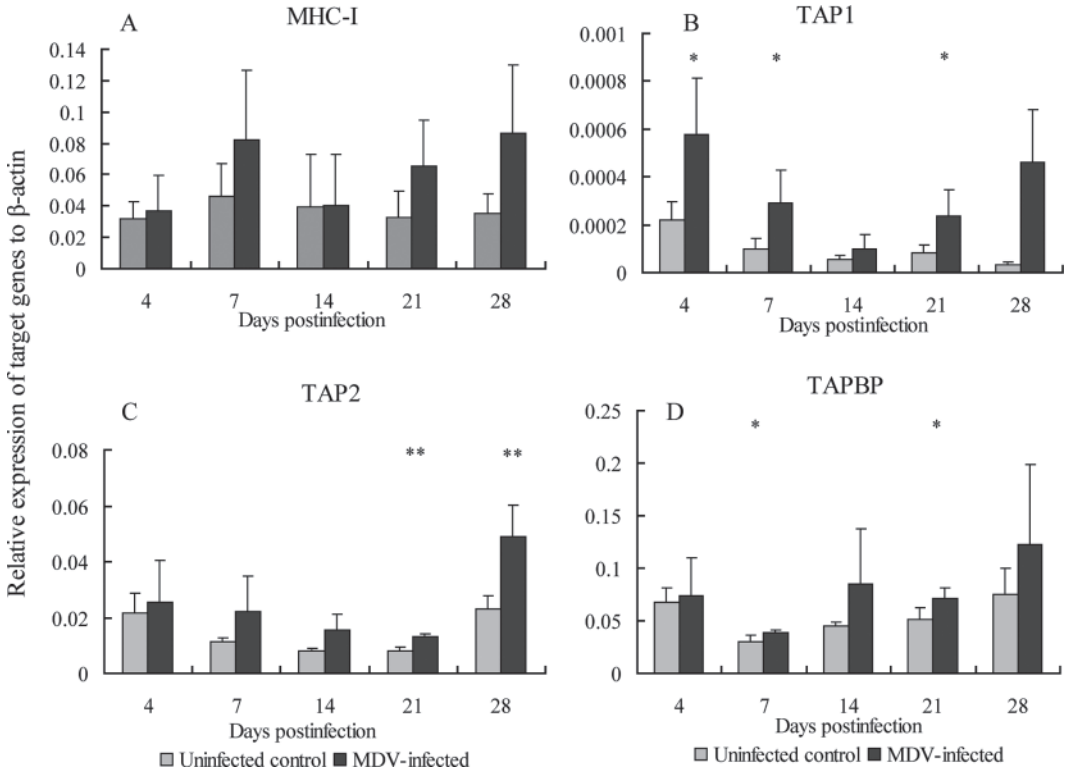


**Figure 1.** Expression of MHC-I pathway genes in spleens of Marek's disease virus (MDV)-infected and uninfected controls. Panels A, B, C, and D show MHC-I, transporter of antigen protein 1 (TAP1), transporter of antigen protein 2 (TAP2), and transporter of antigen protein-binding protein (TAPBP) mRNA expression in spleen, respectively. Groups are as follows: MDV-infected chickens and age-matched controls, sampled on 4, 7, 14, 21, and 28 days postinfection. Target gene expression is presented relative to β-actin expression and normalized to a calibrator. Error bars represent SEM. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.

The expression of the MHC-I gene in MDV-infected chickens is 2 times greater than uninfected age-matched controls during the cytolytic phase of infection (Heidari et al., 2008). Abdul-Careem et al. (2008) proposed that expression of the MHC-I α-chain gene increases in feather pulp at 10 and 14 d p.i. It indicated that the host seemed to enhance cell-mediated immunity by upregulating MHC-I gene expression, which might be relevant to IFN (Sarson et al., 2008).

The expression of TAP1, TAP2, and TAPBP genes in spleen showed a similar trend as MHC-I. The expression of the TAP1 gene (Figure 1B) in spleens of MDV-infected chickens was higher than uninfected controls at 4, 7, and 21 d p.i. ($P < 0.05$). The transcripts of the TAP2 gene (Figure 1C) were higher in spleens of MDV-infected than uninfected chickens at 21 and 28 d p.i. ($P < 0.01$). In chicken, TAP1 and TAP2 genes are in opposite transcriptional orientation and their promoter is situated between them. There are many putative transcription factor-binding sites in there, such as X/X2, Y and S boxes, and the IFN response element, which are in common with the promoter regions of MHC class I and β2 microglobulin. Additionally, both TAP1 and TAP2 can be upregulated by IFN-γ in a similar manner to mammalian TAP genes (Walker et al., 2005). The induction for TAP genes is faster than MHC-I genes (Epperson et al., 1992; Min et al., 1996). Therefore, higher expression of TAP genes in our study might result from IFN-γ, which has been reported increased in MDV infection (Morgan et al., 2001; Sarson et al., 2006, 2008; Abdul-Careem et al., 2008; Heidari et al., 2008).

Similarly, the expression of the TAPBP gene (Figure 1D) was also higher in spleens of MDV-infected chickens than uninfected controls at 7 and 21 d p.i. ($P < 0.05$). The TAPBP gene as another vital member of the MHC-I pathway encodes a transmembrane protein with a probable ER retention signal, bridging the class I α molecule to TAP (Momburg and Tan, 2002; Bangia and Cresswell, 2005). It is required for successful peptide loading and stabilization of the peptide-MHC complex in the ER (Park and Ahn, 2003). In chicken, an alternative encoding production of the TAPBP gene, 8.4 protein, is remarkably similar to mammal tapasin protein (Frangoulis et al., 1999). The TAPBP gene can be upregulated by the IFN-signaling cascade in response to viral infection in mouse (Abarca-Heidemann et al., 2002). Thus, we speculated that expression of TAPBP in our study was enhanced by IFN.

Based on the observations mentioned above, we can conclude that key components in the MHC-I pathway, including MHC-I, TAP1, TAP2, and TAPBP, exhibited upregulated expression patterns in the spleen after MDV infection, which was presumably mediated by IFN (Sarson et al., 2008). It was also revealed that anti-MDV host response was generated in the spleen after MDV infection.

### *MDV Interference with Multiple Components in MHC-II Pathway*

In this experiment, we detected the expression of classical MHC-II β chain gene (BLB2) and 3 nonclassical MHC-II genes (DMA, DMB1, and DMB2) for the first time. The expression of genes in the MHC-II pathway showed a decreased trend at all time points in spleens of MDV-infected chickens with comparison to uninfected controls. The expression of the BLB2 gene (Figure 2A) was lower in spleens of MDV-infected than uninfected chickens throughout and was significant at 28 d p.i. ($P < 0.05$). This was in agreement with previous studies; the expression of MHC Ii chain is decreased 2-fold at 7 and 14 d p.i. in chicken spleen after MDV infection (Sarson et al., 2006). Expression of the MHC-II β chain (BLB) gene is downregulated at multiple sampling points in spleens of B19 and B21 lines of chicken using microarray (Sarson et al., 2008). Heidari et al. (2008) observed that expression of the MHC-II gene is downregulated in spleens of MDV-infected chickens at 5 and 15 d p.i. by real-time PCR. Moreover, Thanthrige-Don et al. (2010) reported that there is a significant downregulation of MHC-II β-chain expression at 4, 7, 14, and 21 d p.i., and other components of the MHC-II heterotrimer (α chain and Ii chain) are also downregulated at 4 and 21 d p.i.

However, in contrast to the observation above, it has been suggested that MDV upregulates the expression of MHC-II in endothelial cells and infiltrating cells such as macrophages and T cells in the central nervous system of infected birds (Gimeno et al., 2001). Niikura et al. (2007) also proposed that MDV directly upregulates the expression level of classical MHC-II Ii chain and cell surface expression in bursa cells of chickens infected with Md11, a very virulent strain of MDV. Aside from the difference of the in vivo and in vitro nature of the previously published research, it is likely that both the genetic background of the host and the strain of the virus affect MDV's influence on MHC expression (Sarson et al., 2008; Thanthrige-Don et al., 2010).

As for nonclassical MHC-II genes, DMA, DMB1, and DMB2 showed similar trends in the spleen as the classical MHC-II gene in this study. The expression of the DMA gene (Figure 2B) was lower in spleens of infected chickens than uninfected controls and significant at 14 and 28 d p.i. ($P < 0.05$). Although the difference was not statistically significant at any time point ($P > 0.05$), expression of the DMB1 gene (Figure 2C) in spleens of MDV-infected chickens did show a consistent lower trend. The expression of the DMB2 gene (Figure 2D) decreased in spleens of infected chickens compared with uninfected controls and was significant at 7 d p.i. ($P < 0.05$). These results indicated that the expression of nonclassical MHC-II genes was interfered by classical MDV.

Generally, reduction of MHC-II expression is recognized as a predominant way of evading the host immune response (Hegde et al., 2003). Herpesviruses produce proteins that target MHC antigen presentation path-

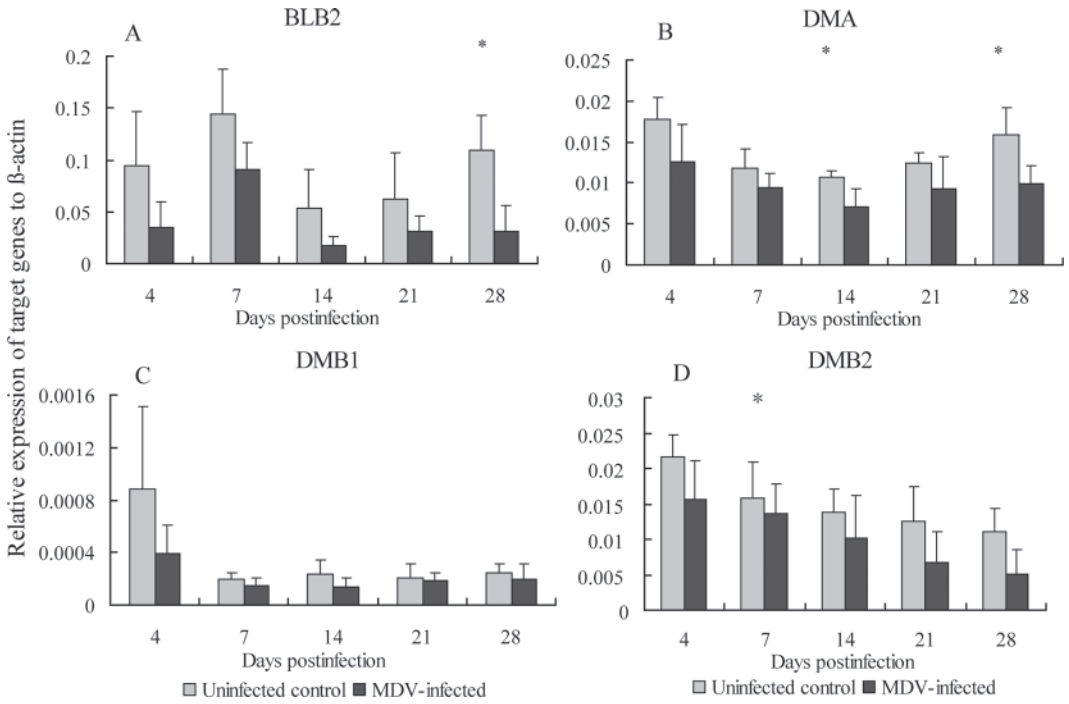


**Figure 2.** Expression of MHC-I pathway genes in spleens of Marek's disease virus (MDV)-infected and uninfected controls. Panels A, B, C, and D show MHC-II β chain major gene (BLB2), DM α chain gene (DMA), DM β chain gene-1 (DMB1), and DM β chain gene-2 (DMB2) mRNA expression in spleen, respectively. Groups are as follows: MDV-infected chickens and age-matched controls, sampled on 4, 7, 14, 21, and 28 days postinfection. Target gene expression is presented relative to β-actin expression and normalized to a calibrator. Error bars represent SEM. $^*P \leq 0.05$.

ways, to escape from host immune response. For example, herpes simplex virus 1 gB binds to the MHC-II α chain to downregulate MHC-II expression, decreasing the ability of the invariant chain to bind to MHC for structural formation and cellular transportation (Neumann et al., 2003). Similarly, in chicken, it is suggested that the MHC-II invariant chain interacts directly with MDV protein repeat long region open read frame 10 (R-LORF10; Niikura et al., 2004). Sarson et al. (2006) proposed that downregulation of MHC invariant chain expression after MDV infection might result from MDV proteins binding to the MHC-II α chain, thereby reducing the ability of the invariant chain to bind to MHC for structural formation and cellular transportation.

In our studies, we found that both the classical MHC-II gene (BLB2) and nonclassical MHC-II genes (DMA, DMB1, and DMB2) exhibited downregulated expression in spleens after MDV infection, which indicated that MDV enabled to interfere with multiple members (i.e., β chain and DM complex) of the MHC-II-mediated antigen presentation pathway.

In conclusion, we detected the expression of TAP1, TAP2, TAPBP, DMA, DMB1, and DMB2 in MDV-infected spleen for the first time. The MHC-I pathway genes exhibited higher transcripts after MDV infection, which indicated MDV stimulated the expression of genes involved in the endogenous antigen presentation pathway to enforce cell-mediated immune response. With respect to MHC-II, we found that nonclassical MHC-II genes exhibited consistent lower expression in spleens of MDV-infected chickens as the classical one, which revealed that MDV interfered with multiple components of MHC-II complex.

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