Microarray Analysis of Host Immune Responses to Marek’s Disease Virus Infection in Vaccinated Chickens

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ABSTRACT. Marek’s disease (MD) is a commercially important disease of chickens caused by MD virus (MDV). Although avirulent MDV strains have been used for vaccination to prevent MD outbreaks, the protective mechanism of the vaccine has not been elucidated. In this study, a comprehensive transcriptional analysis using microarray was conducted in MDV-infected chickens with and without vaccination at 7 and 21 days post-infection (dpi). The data suggested that the expression of T cell receptor (TCR) 1-related genes was up-regulated in vaccinated-challenged compared to unvaccinated-challenged chickens during the latent phase of infection. Consistently, this induction was confirmed by quantitative PCR. Flow cytometric analysis revealed that most of TCR1+ cells expressed CD8α chain brightly. The number of this subpopulation was significantly and specifically increased in vaccinated-challenged chickens at 21 dpi compared to unvaccinated-challenged chickens, though it was not the major population in spleen of chickens. The number of CD8αhigh TCR2+ cells, the major subpopulation of chicken CD8αhigh cells, was increased in vaccinated chickens with or without challenge compared to unvaccinated control chickens. These data suggested that both CD8αhigh TCR1+ and CD8αhigh TCR2+ cells could be induced by the vaccination. It is also possible that CD8αhigh TCR1+ cells might be primed by the vaccination and specifically induced by the challenge with virulent strain of MDV during the latent phase of infection. Thus, CD8αhigh TCR1+ cell population is probably one of the key factors involved in the protective mechanism induced by a vaccine strain, CVI988.

KEY WORDS: CD8α, Marek’s disease, microarray, TCR1, vaccine.

Marek’s disease (MD), caused by Marek’s disease virus (MDV), is a lymphoproliferative disease resulting in the formation of malignant T cell lymphoma in visceral organs of chickens [14]. Strains of MDV, an avian herpesvirus, are divided into 3 serotypes; serotype 1 (MDV-1) is oncogenic in chickens while serotypes 2 (MDV-2) and 3 (herpesvirus of turkey [HVT]) are nonpathogenic. Infection with virulent strain of MDV-1 causes an early cytolytic infection initially in B lymphocytes at 3–7 days post-infection (dpi), and T lymphocytes are activated against that infection [3]. Subsequently, MDV invades activated T cells, and establishes latency after about 6–7 dpi. [38]. On the 3–4 weeks post-infection, MDV is reactivated, and enters the phase of pathogenesis. Mechanisms underpinning the establishment and maintenance of latency have not been elucidated, but it has been suggested that host immune responses are the factors for them [13, 35].

MD has been prevented by the vaccination with avirulent strains of MDV in poultry industry. Attenuated MDV-1 strains, as well as MDV-2 and HVT, are used as monovalent or bivalent vaccines. CVI988, a strain of naturally attenuated MDV-1, is considered to be the most protective vaccine currently available and is used in many countries [14, 42]. However, the details of preventive mechanism by these vaccines are still unknown. MD vaccines can inhibit the onset of clinical MD but not prevent the invasion of virulent strain [9]. The propagation of avirulent vaccine strains of MDV in vivo are very low compared to virulent strains [22]. Neutralization antibodies are induced by the vaccination but are not essential for the inhibition of viral propagation [9]. It is necessary to elucidate how the vaccine works because the continuous increase in MDV virulence has prompted concern that the current available vaccines would later lose their efficacy in controlling MD [10]. Some previous studies have reported cell-mediated immunity (CMI), which is effective to prevent MDV propagation, is activated in chickens vaccinated with MDV-2 and HVT [1, 18, 29]. On the contrary, there are few reports to mention the host immune responses induced by the vaccination with CVI988 [19, 27, 28].

Comprehensive analysis of gene expression using microarray can give us important information. Recently, microarray analysis in chicken embryo fibroblasts, peripheral blood leukocytes or splenocytes of chickens infected with HVT or MDV were reported [20, 21, 23, 26, 31, 32]. However, there was no microarray report about the differences in the host immune responses induced by MDV infection between vaccinated and unvaccinated chickens. We hypothesized that the patterns of gene expressions are different between MDV-infected chickens with and without the vaccination of CVI988. In the present experiment, gene expressions in MDV-infected, vaccinated-challenged and only vaccinated chickens were analyzed by cDNA microarray. Quantitative PCR and flow cytometric analysis was also conducted to confirm the microarray result. Our findings suggest a potential effect of T cells expressing TCR1 against MDV-infection.
MATERIALS AND METHODS

Chickens: Neonatal male White Leghorn chickens were purchased from Hokuren Co. Ltd (Sapporo, Japan) and divided into 4 groups randomly. Each group consisted of 35 chickens. The flock was free of common poultry diseases and not vaccinated against MDV. Chickens were maintained in an isolator per group. Feed and water were provided ad libitum.

Viruses: A strain of very virulent MDV1, RB1B [33], was obtained from chicken kidney cell culture of experimentally infected chickens. A vaccine strain of MDV1, CVI988, was purchased from Gehn Corp. Inc. (Gifu, Japan). Both strains were propagated in chicken embryo fibroblasts (CEF), and virus titers were determined by plaque assays as described previously [43]. These infected CEF were used for the viral inoculation of chickens.

Experimental design: Chickens in Group A were infected on day 5 post-hatch with 2,000 PFU of a virulent strain, RB1B, by intraperitoneal (i.p.) injection. Chickens in Group B were vaccinated at one day of age with 1,000 PFU of an avirulent strain, CVI988, by i.p. injection, and challenged with 2,000 PFU of RB1B strain at 4 days after vaccination. Chickens in Group C were vaccinated only with the same vaccine at one day of age, and chickens in Group D were untreated as controls. Spleen tissues were collected from euthanized chickens in each group on the 7 and 21 dpi. Spleen cells were isolated by density gradient centrifugation on Percoll (GE Healthcare UK Ltd., Amersham Place, England). This study was conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of Hokkaido University, Japan.

cRNA preparation and microarray hybridization: Total RNA was extracted from isolated spleen cells on the 7 and 21 dpi using TRIZOL™ reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA), and stored at −80°C until use. Agilent chicken 4 × 44K oligo DNA microarray slides (Agilent Technologies, Inc., Santa Clara, CA) were used for the analysis of gene expression in splenocytes of chickens infected with MDV, and all samples were tested in duplicate. Microarray process was done according to the Agilent protocol. Briefly, cDNA was synthesized from a 500 ng aliquot of total RNA and transcribed into cRNA labeled with cyanine 3 using the Low RNA Input Fluorescent Linear Amplification Kit PLUS (one-color) (Agilent Technologies). Labeled cRNA was purified with RNeasy Mini columns (Qiagen, Valencia, CA). The quality and quantity of each cRNA sample were assessed with the Agilent Bioanalyzer 2100 and RNA 6000 Nano LabChip Kit (Agilent Technologies) and NanoDrop ND-1000 spectrophotometer measurement (Thermo Fisher Scientific, Waltham, MA). Labeled cRNAs were used for hybridization using the Gene Expression Hybridization Kit (Agilent Technologies). Arrays were incubated at 65°C for 17 hr in Agilent’s microarray hybridization chambers. Then they were washed according to the Agilent protocol and scanned at 5-μm resolution using the Agilent Microarray Scanner. Data were extracted using the Feature Extraction Software (version 9.1, Agilent Technologies) and saved as the TIFF format.

Microarray data analysis: Data were normalized and analyzed using the GeneSpring GX Software version 7.3 (Silicon Genetics, Redwood City, CA). Normalized data were clustered based on Gene Ontology, and genes belonging to the group of “host-pathogen interaction” were selected for comparison. Differential expression of a given gene within the experiment was obtained if a 2-fold or greater difference was observed in either of the 2 comparisons. Statistical analysis was done using the Student’s t-test, and data were considered significantly different if probability values of P<0.05 were obtained.

Real-time RT-PCR: By real-time RT-PCR, the expression level of the TCR gamma and delta chain (TCRGV and TCRDV) mRNA in splenocytes on the 7 and 21 dpi was quantified. The sequences of each primer were as follows: TCR gamma chain (foward-5'-TTTTTCATAGGGTCAGTAGCAGTAG-3', reverse-5'-GGCACAGTAGTAAGTAGA GGCGATCA-3'); TCR delta chain (forward-5'-TCGAGTGTTGTCCCTCTATATACC-3', reverse-5'-AGTAGGGCTGAGTTGTTGGAAG-3', reverse-5'-AGGGCATCAGGGACAGCACA-3'). All primers were BLAST-searched for chicken DNA sequences available in GenBank to ensure specific amplification, and were synthesized by Hokkaido System Science Co., Ltd. (Sapporo, Japan). Real-time RT-PCR assay was performed using the Light cycler™ (Roche Diagnostics, Mannheim, Germany). Two microgram of each RNA sample were treated with DNase I (Invitrogen, Carlsbad, CA) to remove residual DNA, and cDNA was synthesized from the RNA samples with moloney murine leukemia virus reverse transcriptase (TAKARA, Shiga, Japan) as directed by the manufacturer. The cDNA template was added to a total volume of 20 μl containing PCR buffer, oligonucleotide primers (at 0.2 μM each of primers), and 2 μl of the LightCycler-Fast Start DNA Master SYBER Green I (Roche Diagnostics, Mannheim, Germany). Serial dilutions of the pGEM-T easy vector (Promega, Madison, WI) containing each target gene (1 × 10⁻⁵ to 1 × 10⁻¹ ng/ml) were used to generate standard curve for quantification. TCR gamma or delta chain mRNA expression was shown as ratio obtained by dividing the concentration of TCR chain mRNA by that of the β-actin mRNA. The specificity of amplification was confirmed by melting point analysis and length of PCR products. Results were expressed in folds of each TCR chain mRNA expressions compared to the control.

Flowcytometric analysis of lymphocyte subset: Freshly isolated splenocytes (1 × 10⁶) were resuspended in PBS supplemented with 1% bovine serum albumin (Sigma), and were stained by one of the combinations of the monoclonal antibodies, CD4-FITC/TCR1-PE, CD8-FITC/TCR1-PE, CD4-FITC/TCR2-PE, or CD8-FITC/TCR2-PE (Southern Biotechnology Associates, Birmingham, AB). As a control, mouse non-immune IgG-FITC and IgG-PE were used.
After 30 min of incubation at 4°C, the cells were washed three times with PBS, and fixed in PBS containing 2% formaldehyde. Stained cells were analyzed by EPICS XL flow cytometry system (Beckman Coulter, Fullerton, CA) with the EPICS EXPO32 ADC software.

Statistical analysis: Data of real-time RT-PCR and flow cytometry were analyzed by one-way analysis of variance followed by the Student’s t-test using the Statcel2 software (OMS, Saitama, Japan). Differences between Groups were considered significant if probability values of \( P<0.05 \) were obtained.

RESULTS

Gene expression profiling of spleen tissue in chickens infected with MDV: A comprehensive profiling of gene expression was performed by microarray analysis of spleen cells from MDV-infected chickens at 7 and 21 dpi. Data were normalized using data from Group D in each schedule and gathered 380 genes belonging to the group of “host-pathogen interaction”, based on Gene Ontology. This raw data presented in Fig. 1 showed that the expression levels of most of the genes in Group B were lower than those of Group A at 7 dpi. However, many of them were increased in Group B at 21 dpi compared to Group A in which the expression levels of most of the genes were very low. To remove insignificant data, we decided to put the significance limit of gene expression level to \( \geq 2 \)-fold and \( P<0.05 \). The numbers of the genes which showed significant differences in the expression levels between Groups (A/B, A/D, B/D and C/D) at each schedule were presented in Table 1a. Most of the genes were highly expressed in Group A compared to Groups B and D at 7 dpi (30 and 22 genes, respectively). On the contrary, 20 out of 21 genes and 23 out of 35 genes were significantly down-regulated in Group A compared to Groups B and D at 21 dpi, respectively. Among these genes, the expression of the granzyme A gene was up-regulated in both Groups A (120.5- and 8.547-fold at 7 and 21 dpi, respectively) and B (20.66- and 4.566-fold at 7 and 21 dpi, respectively) compared to Group D on both schedules (data not shown). Interestingly, the expressions of \( \gamma \delta \) T cell receptor (TCR1)-related genes were significantly up-regulated in Group B while their expression levels remained low in Group A at 21 dpi (Table 1b), though they were down-regulated at 7 dpi in both Groups A and B compared to Group D (data not shown).

Quantification of expression levels of TCR1 relating genes using quantitative RT-PCR: In the microarray data, only the genes related to TCR1 commonly showed significant differences between groups during the experimental period. To confirm them, the expression levels of gamma and delta chain genes of TCR were quantified using real-time RT-PCR at 7 and 21 dpi (Fig. 2). Three samples per group were used for this experiment. In Group A, the expression levels of TCR gamma chain did not show significant change compared to those in controls at 7 and 21 dpi (1.01-fold ± 0.642 and 1.37-fold ± 0.547, respectively) (Fig. 2b). However, the expression levels of delta chain in Group A were down-regulated at 7 and 21 dpi (0.587-fold ± 0.303 and 0.572-fold ± 0.176, respectively) (Fig. 2a). Group B chicks showed slight increase in gamma and delta chain gene expression at 7 dpi (1.83-fold ± 0.606 and 1.34-fold ± 0.501, respectively). At 21 dpi, their expressions was further increased (3.28-fold ± 0.938 and 2.03-fold ± 0.78, respectively), and the levels were significantly higher than Group A (\( P<0.05 \)) (Fig. 2a and b). The expression levels of these genes were slightly increased in Group C at 7 and 21

![Fig. 1. Box plots of the intensity log ratio to control. The changes in the expression levels of genes belonging to “host-pathogen interaction” (based on GO) in MDV-infected chickens at 7 (dark gray) and 21 (pale gray) dpi were analyzed by microarray. These data were normalized by control in each specific time and represented as box plots by Gene Spring software.](image-url)

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a) Genes concerning “host-pathogen interaction” (based on GO) were gathered and analyzed with Gene Spring software to remove insignificant data.

b) (+) indicates the numbers of the genes upregulated, and (–) indicates genes downregulated in the left group compared to the right group.
Flow cytometric analysis of splenocyte subpopulations in chickens infected with MDV: We analyzed the subpopulation of TCR1+ cells in each group at 7 and 21 dpi using the flow cytometry as well as analysis of gene expression. The results showed that most of TCR1+ cells highly expressed the CD8α molecule, known as CD8αhigh cells (Fig.3a). The number of CD8αhigh TCR1+ cells was significantly larger in Group B compared to Group C at 7 dpi (P<0.01) (Fig. 3b). Although no difference was observed between the number of CD8αhigh TCR1+ cells in Groups A and B at 7 dpi, the number was significantly increased in Group B than Group A at 21 dpi (P<0.05) (Fig. 3a and b). On the other hand, the major type of TCR expressed on CD8αhigh cells was TCR2, a homologue of mammalian αβ TCR, and the number of CD8αhigh TCR2+ cell was significantly increased in Group B compared to Groups C and D at 21 dpi (P<0.05) as well as in Group C compared to Group D only at 21 dpi (P<0.05) (Fig. 3c). Contrary to the CD8αhigh TCR1+ cell subset, no significant difference was observed between the percentage of CD8αhigh TCR2+ cell in spleen of Groups A and B during the experimental period (Fig. 3c). The main subset of TCR expressed on CD4+ cells was TCR2, and no significant difference was shown in this subpopulation between the groups at 7 and 21 dpi (Fig. 3d).

DISCUSSION

MD vaccine can inhibit the propagation of virulent MDV at early latent phase and maintain the latency. Previously, it has been strongly suggested that host immune responses, especially CMI, are important for the protective effect of the vaccine [1, 18, 29]. The network of immune responses in chickens infected with MDV could be very complicated, and thus, comprehensive analysis of them is necessary to clarify the protective mechanism of MD vaccine. In the present study, by microarray, comprehensive transcriptional analysis of the genes related to immune responses was conducted in chickens infected with MDV. The results showed that, while the expression levels of various genes related to “host-pathogen interaction” were increased in chickens challenged with virulent MDV at 7 dpi, the early latent phase, many of them were decreased at 21 dpi. On the contrary, many genes in vaccinated-challenged chickens were at higher levels of expression compared to control chickens at 21 dpi, suggesting that the immune responses against MDV infection is still active during the latent phase and keeps virulent MDV to latency. MD vaccine might contribute to the prevention of immune suppression in chickens infected with virulent strain of MDV. Moreover, the genes related to TCR1 showed significant differences between MDV-challenged chickens with and without vaccination. Their expressions were increased in spleen of vaccinated-challenged chicken at 21 dpi but not in unvaccinated-challenged chickens. Thus, we particularly focused on the
dynamics of TCR1+ cells.

Avian T cell populations are divided into 3 groups based on types of TCR. The first is TCR1+ cell subset which is a homologue of mammalian γδ T cell. Although the γδ T cell is a minor population in peripheral blood or spleen of humans and mice, it is one of the major circulating T cell subsets in chickens [11, 39]. The other two are TCR2+ and TCR3+ cell subsets, homologues of mammalian αβ T cell.

While the TCR2+ cell expressing Vβ chain is a major subset of peripheral T cells of chicken, TCR3+ cell expressing Vβ chain is comparatively minor [15, 16]. The detailed function of TCR1+ cells is not clarified in chickens. The γδ T cells in mammals have been reported to display anti-viral or anti-tumor functions [5, 17, 25, 36]. Although chicken TCR1 cells were also suggested to show cytotoxic effects as well as NK cells [37], the majority of cytotoxic lymphocytes (CTLs) has been reported to be TCR2+ cells [40], and only few reports were published about the function and kinetics of TCR1+ cells in chickens infected with various pathogens including MDV [4, 6, 8, 29]. In our experiment, although the sample number was small, microarray analysis suggested that the effect of MD vaccination may include the induction of TCR1+ cells. To confirm this, when we quantified the expression levels of TCR1-related genes in splenocytes by real-time RT-PCR, higher levels of expression of both TCRDV and TCRGV genes were observed in vaccinated-challenged chickens compared to challenged-chickens. These results suggested that TCR1+ cells, which may play a role in the response to MDV infection, are induced by the vaccination with CVI988. Alternatively, the vaccine might have a priming effect on TCR1+ cells, and the subsequent challenge might induce TCR1+ cells, because the expressions of TCRDV and TCRGV genes were significantly increased in vaccinated-challenged chickens but not in chickens only vaccinated compared to control.

With the flow cytometric analysis, we observed that the major population of TCR1+ cells in splenocytes of chickens brightly expressed CD8α chain. CD8α+ T cells in chickens are divided into two subpopulations [7, 24]. The one expressing CD8α chain comparatively weakly is CD8αdim T cells. CD8αdim T cells are suggested to function as memory cells, and the CD8 complex of this subpopulation consists of homodimer chains, CD8αα. The other subpopulation expresses CD8α chain brightly (CD8αhigh), which mainly forms heterodimer complex with CD8β chain. Previous report showed CD8β chain enhanced specific TCR-mediated responses of CD8+ T cells in human [41]. This subpopulation has been reported to also function as CTLs in chicken [6, 7, 24].

CD8+ CTL is an effector subset against MDV infection [27, 28]. In chickens vaccinated with HVT, the increased number of circulating CD8+ T cells has been observed [30]. Moreover, some previous reports strongly suggested that CVI988 could induce activation of CD8+ T cells which have anti-viral and anti-tumor functions [19, 27, 28] though the subpopulation of CD8+ T cells were not estimated. In the present study, we observed that the number of CD8αhigh TCR1+ cell was significantly decreased in spleens of chickens infected with virulent MDV at 21 dpi compared to vaccinated-challenged chickens. This result indicated that CD8αhigh TCR1+ cell was one of the important effector subgroups in chickens vaccinated with CVI988. Although the detailed mechanisms of this induction were still unknown, IFN-γ might be one of the important factors, because CVI988 induced the expression of the IFN-γ gene in periph-

Fig. 2. The expressions of the TCRDV and TCRGV mRNA in splenocytes from chickens experimentally infected with MDV at 7 and 21 dpi (n=3 samples per each time point). The results are presented in means and SEMs in each schedule by group. The extent of gene expression (-fold) was calculated by dividing the value of each sample by that of the value of each control. Chickens were infected with RB1B (Group A; black bar), CVI988 (Group C; open bar), or both CVI988 and RB1B (Group B; gray bar). The significant differences were determined by the Student’s t-test between Groups (* P≤0.05, ** P≤0.01).
eral blood and feather follicle epithelium, and increased the number of CD4+ T cells including Th1 cells, the inducer of CD8+ T cells [2, 19]. Though the direct effect of CD8αhigh TCR1+ cells against MDV-infected cells remains to be confirmed, the present study suggests a potential activation of CD8αhigh TCR1+ cells in vaccinated-challenged chickens. Microarray data also showed an increase in the expression of the granzyme A gene in splenocytes of vaccinated-challenged chickens compared to control chickens. Granzyme A is known as CTL-associated serine esterase 3, and is produced by CTL and NK cells. CD8αhigh TCR1+ cells might express granzyme A as CTLs to inhibit the reactivation of MDV. However, the expression level of granzyme A was more increased in unvaccinated-challenged chickens at 7 and 21 dpi and vaccinated-challenged chickens at 7 dpi. Thus, granzyme A may be important for the early cytolytic infection or the reactivation of MDV, which is reported to start reactivation from latency at 3–4 weeks post-infection.

Although CD8αhigh TCR1+ cells are suggested to be a candidate to inhibit MDV propagation or transformation of T cells by MDV, the number of CD8αhigh TCR2+ cells were also increased in vaccinated-chickens with or without challenge compared to control chickens at 21 dpi in the present study. These data suggested that CD8αhigh TCR2+ cells were induced by the vaccination and play a role in the prevention of MD. In fact, Omar et al. [29] reported that the TCR2+ cells are effective against MDV-infected cells, and TCR1+ cells are slightly effective in vaccinated chickens. However, this previous study used SB-1 vaccine which belongs to MDV2 and the cytotoxic function was only observed in vitro. In our data, the population of CD8αhigh TCR2+ cells showed no significant difference between unvaccinated-challenged and vaccinated-challenged chickens, suggesting that this subset was induced by the infection with virulent MDV as well as the vaccination. Thus, further studies are necessary to clarify the role of CD8αhigh TCR1+ cells in chickens.

Fig. 3. The subpopulations of CD8α+ T cell (n=6–9 per each group) and CD4+ T cell (n=3–6 per each group) in splenocytes from chickens experimentally infected with MDV at 7 and 21 dpi were analyzed by flow cytometry. (a) The frequencies of splenic CD8αhigh TCR1+ cells in a virulent MDV-infected chicken (Group A; left panel), and a vaccinated-challenged chicken (Group B; right panel) at 21 dpi, are represented by dot plots. Gated area indicates the subpopulation of CD8αhigh TCR1+ cells. (b-d) The percentages of (b) CD8αhigh TCR1+ subset, (c) CD8αhigh TCR2+ subset, and (d) CD4+ TCR2+ subset in splenocytes of chickens were presented in means and SEMs in each schedule by group. Chickens were infected with either RB1B (Group A; black bar), CVI988 (Group C; pale gray bar), or both CVI988 and RB1B (Group B; dark gray bar). Uninfected chickens were used as controls (Group D; open bar). The significant differences were determined by the Student’s t-test between Groups (* P<0.05, ** P<0.01).
cells to prevent MD in MDV-infected chickens with or without vaccination.

The other possible effect of CD8αhigh TCR1+ cells against MDV infection is that they change the host internal environment. We observed that the main subpopulation of TCR1+ was CD8αhigh cells in both chickens infected and uninfected with MDV, and the increase in this subpopulation could decrease the effect of transformation by MDV because its target for transformation is CD4+ T cells. The MD cell lines generated from natural MD lymphoma are known to express either TCR2 or 3 but not TCR1 [34]. Moreover, the percentages of both CD8α and TCR1 cells were very low in MD lymphoma although that of CD4+, TCR2 and TCR3 cells were more frequent [12]. Lee et al. [22] suggested that the cell tropism of attenuated MDV1 (CVI988) was different from that of MDV2 or virulent MDV1. In their reports, the low level of CVI988 could be detected from both of CD4+ and CD8+ T cells equally at 7 dpi, and the level was almost same with virulent MDV1 in CD8+ T cells. We observed the major subset of TCR1+ cells in spleen were CD8αhigh cells, suggesting that almost all TCR1+ cells can be infected with CVI988 at the low level. Thus, the increase in the number of CD8αhigh TCR1+ cells can inhibit indirectly the onset of MD because this subset is not the target for transformation by virulent MDV1. The population of CD4+ TCR2+ cells showed no difference between groups. However, it was shown that the number of latently MDV-infected cells was very low in the CD4+ cell population of vaccinated-challenged chickens [22], and thus, the reactivation of MDV could be a very rare event occurred in vaccinated-challenged chickens. On the other hand, it was also suggested that MD vaccine enhances the host innate immunity effective to prevent the MDV propagation. Thus, other subsets as well as CD8αhigh TCR1+ cells should contribute to the prevention of propagation and reactivation of MDV in each phase of infection.

In conclusion, present study suggests a potential role of CD8αhigh TCR1+ cells on the protective effect induced by the vaccination with CVI988. This suggestion would be important to elucidate the detailed protective mechanisms induced by MD vaccine. Further experiments are required to clarify the detailed function of CD8αhigh TCR1+ cells against MDV-infection.

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