Kinetic Analysis of T Cells and Antibody Production in Chickens Infected with Marek's Disease Virus

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ABSTRACT. In chickens inoculated with a Marek's disease (MD) vaccine and subsequently with virulent MD virus (MDV), $CD4^+$ T cell population was drastically decreased following a transient increase at 21 days after hatching (16 days after MDV infection). To elucidate the immune response after the decrease of $CD4^+$ T cell population, the antibody production against sheep red blood cells (SRBC) was examined in these chickens. Chickens challenged with a virulent MDV after MD vaccination produced lower titers of anti-SRBC antibody than untreated control chickens. Antibody production against SRBC was also lowered in vaccinated chickens or chickens challenged with a virulent MDV.—KEY WORDS: helper T cell, Marek's disease virus.
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Marek's disease (MD), caused by a highly cell-associated herpesvirus, is a lymphoproliferative disease of chickens. After the introduction of vaccines developed from apathogenic strains of MD virus (MDV), MD became the first neoplastic disease which is effectively controlled by vaccination. Though cell-mediated immunity, including cytotoxic T lymphocytes, has been suggested to play an important role in that mechanism [11, 12], the exact protection mechanism(s) of MD vaccines has not been elucidated. In addition, the function of T cells of chickens has been poorly understood compared to the B cell system of chickens. Therefore, it would be important to characterize the function of T cells in MD-vaccinated chickens. We previously reported the role of $CD4^+$ T cells on antibody production by using CD4-depleted chickens. This observation suggests that $CD4^+$ T cells in chickens have the helper function for the differentiation of B cells to antibody-producing cells, comparable to those in mammals [15]. MDV infection also causes the decrease of the $CD4^+$ T cell number in chickens, which are target cells for MDV transformation [8]. Since several studies have indicated that MDV infection depresses the T cell function [1, 2], we analyzed more detailed kinetics of $CD4^+$ T cells in MDV-infected chickens. In addition, the correlation between the decrease of $CD4^+$ T cells and antibody production in those chickens was studied.

Chickens (Sherver 288 White Leghorn) were vaccinated intramuscularly with an attenuated strain of MDV, CVI988 [10] (1000 PFU; Kyoritsu-shoji Co., Ltd., Japan), at 1 day after hatching (DAH). Then, vaccinated chickens were challenged intraperitonealy with a highly virulent strain of MDV, Md5 (7200 PFU), at 5 DAH. Peripheral blood mononuclear cells (PBMC) were prepared from these chickens, and subjected to flow cytometric analysis (Profile, Coulter Corp., Hialeah, FL). Two T cell subset-specific MAbs, CT4 (anti-CD4, a gift from Dr. C-L. H. Chen, University of Alabama, Birmingham, Alabama) [4] and Lc-4 (anti-CD8) [7] were used in this analysis. The results are shown in Fig. 1. More than 20% of $CD4^+$ T cells were observed in untreated control chickens throughout this experiment (Fig. 1). A drastic decrease of $CD4^+$ T cells was observed following a transient increase in vaccinated chickens challenged with strain Md5. Recently, it was found that the decrease of $CD4^+$ T cells is due to apoptosis though the exact mechanism remains unknown (Morimura *et al.*, submitted). Chickens inoculated with Md5 alone also displayed a slight decrease of $CD4^+$ T cell population, but the population then increased gradually. In contrast, the remarkable change was not detected in $CD8^+$ T cells of all groups (data not shown).

Previously, we found that chicken $CD4^+$ T cells have helper function comparable to those in mammals. Remarkable reduction of anti-SRBC antibody production was observed in CD4-depleted but not CD8-depleted chickens [15]. Since, in MD-vaccinated chickens inoculated with Md5, a drastic decrease of $CD4^+$ T cell population was observed, we next examined the effect of the decrease of $CD4^+$ T cell population on antibody production in these chickens. Each group of chickens, vaccinated and/or challenged with MDV, was intramuscularly immunized with 2×10^8 cells/0.1 m*l* of SRBC mixed with either complete Freund's adjuvant (for the first immunization) or incomplete Freund's adjuvant (for boosters). The chicken were immunized at 7, 21, 28 and 35 DAH. Titers of anti-SRBC antibodies were monitored by enzyme-linked immunosorbent assay (ELISA) using soluble SRBC membrane antigen as described previously [5]. The results are shown in Fig. 2. Throughout the experimental period, titers of anti-SRBC antibodies in MD-vaccinated chickens challenged with Md5 were lower than those in untreated control chickens although no statistical significance was shown. OD_{415nm} values of anti-SRBC antibodies ranged between 0.25 and 0.30 in vaccinated chickens challenged with Md5 while those ranged between 0.35 and 0.45 in control chickens or in vaccinated chickens. In chickens

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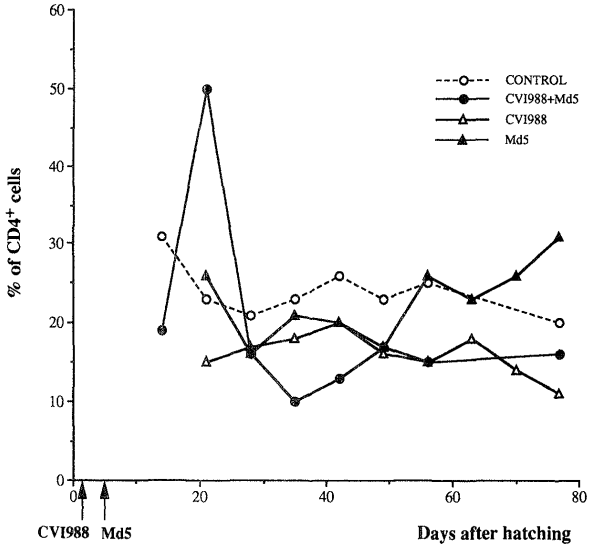

Fig. 1. Kinetic analysis of CD4+ T cell of PBL in the chickens after MD vaccination followed by the MDV challenge. The ratio of CD4+ T cells in PBL was measured by flowcytometry. PBL used in this analysis were obtained from chickens in the control, vaccinated with CVI988 at 1 DAH, challenged with Md5 at 5 DAH, and challenged with Md5 after MD vaccination. Inoculation of MDV (CVI988 and/or Md5) were indicated with arrowheads.

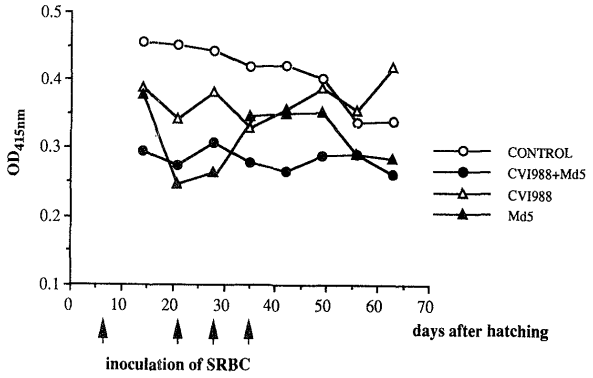

Fig. 2. Titers of anti-SRBC antibody in chickens vaccinated with CVI988 and challenged with Md5. Chickens were inoculated with MDV, and then immunized intramuscularly with SRBC (2×10^8 cells) at 7, 21, 28 and 35 DAH. Serum samples were obtained from these chickens at 14, 21, 28, 35, 42, 49, 56 and 63 DAH. Antibody titers against SRBC were determined by ELISA, and expressed as OD_{415nm}. open circle, uninfected control chickens; closed circle, chickens vaccinated with CVI988 and inoculated with Md5; open triangle, chickens vaccinated with CVI988; closed triangle, chickens inoculated with Md5.

challenged with Md5, titers of anti-SRBC antibodies were transiently decreased at 21 DAH, and then recovered until these chickens developed clinical signs of MD at about 50 DAH.

It is known that MDV causes cytolytic infection of B cells during the early phase of MDV infection [13, 14], and subsequently infects activated T cells, which are the target for both latent infection and transformation [11, 12]. During the cytolytic infection of B cells, humoral immunity is low, but this immunity recovers in the latent phase [3, 9]. This is consistent with our results: a transient decrease and the subsequent recovery of antibody production were observed in chickens infected with MDV (Fig. 2). In the case of immunization with a T-dependent antigen, SRBC, antibody titers were lower in chickens inoculated with MD vaccine and challenged with MDV than in control chickens. On the other hand, Kermani-Arab *et al.* [6] reported that the antibody response to SRBC in MDV-infected chickens are almost same as that in normal chickens. This difference might be due to the ages and/or lines of chickens used in the experiments. Moreover, the reduction of antibody production demonstrated in this study may be caused by the decrease of CD4+ T cells or the anergy of these cells although the direct proof of this hypothesis was not shown in the present study. However, based on the fact that antibodies are detectable throughout this experiment, the remaining CD4+ T cells may have the function to differentiate B cells into antibody-producing cells, similar to Th2 cells in mice. This also may be the reason why vaccinated chickens can survive without developing immunodeficiency although CD4+ T cell population was decreased by MD vaccination. The effect(s) of the decrease of CD4+ T cell population on other immune functions, including the rejection of tissue transplantation, is now under investigation.

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