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Citation	Japanese Journal of Veterinary Research, 41(1), 34-35
Issue Date	1993-05-27
Doc URL	<a href="http://hdl.handle.net/2115/2426">http://hdl.handle.net/2115/2426</a>
Type	bulletin (article)
File Information	KJ00002377639.pdf



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## INVOLVEMENT OF CD4<sup>+</sup> T CELL IN THE PROTECTIVE EFFECTS OF MAREK'S DISEASE VACCINES

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Marek's disease (MD) is a lymphoproliferative disease characterized by T cell lymphoma formation and peripheral nerve enlargement, which can be prevented by vaccination. MD is therefore often considered to be a model for studying antitumor immunity. Although the vaccines were shown to initiate apparently both humoral and cellular immune responses in chicken, the exact mechanism of MD vaccines remains largely unknown. Kudo et al. reported that none of the chickens in which CD4<sup>+</sup> T cells were depleted by thymectomy and injecting anti-chicken CD4 monoclonal antibody (Lc-6: mouse IgM), showed visceral lymphomas after infection with a virulent MD virus (MDV) (strain Md5), suggesting that CD4<sup>+</sup> T cells were involved in the lymphoma formation of MD.

In order to confirm the previous observations and to elucidate the roles of CD4<sup>+</sup> T cells in the protective effects of MD vaccines, CD4<sup>+</sup> T cell in peripheral blood of chickens were completely deleted by injecting anti-CD4 antibody (CT4: mouse IgG1) which has a longer half-life in days than Lc-6. In control and CD8-depleted groups, most of the chickens showed either visceral lymphoma or peripheral nerve enlargement after challenge with strain Md5. In CD4-depleted group, however, 3 of 4 chickens had no MD lesions and only one chicken developed visceral lymphoma. Flow cytometric analysis revealed that the lymphoma cells of this chicken had no T cell markers. These results indicated that the complete depletion of CD4<sup>+</sup> T cells from chickens could prevent MD lesions, including visceral lymphoma and peripheral nerve enlargement. Moreover, non-CD4<sup>+</sup> T cell may become the target cells of MD lymphoma in particular conditions such as the complete depletion of CD4<sup>+</sup> T cells.

The change of percentage in the T cell subsets in PBL of chickens after MD vaccination and challenge with strain Md5 was examined. In chickens only vaccinated with strain CVI988 (CVI988 group) one day after hatching (DAH), a gradual decrease of CD4<sup>+</sup> T cells was observed followed by a transient increment at 21 DAH. This phenomenon was drastic in the chickens challenged with strain Md5 after vaccination (CVI988-Md5 group). Flow cytometric analysis by using anti-chicken T cell receptor (TCR) monoclonal antibodies also revealed the expression of V $\beta$ <sub>1</sub>- $\alpha$   $\beta$  TCR on CD4<sup>+</sup> T cells decreased by MD vaccination. Furthermore, total DNA of PBL in the chickens of the CVI988 group showed an apoptosis-specific pattern of nucleosomal laddering at 21 and 26 DAH by agarose gel electrophoresis. This laddering was absent in DNA

from PBL of control chickens. This suggests that MD vaccines induce a decrease of  $CD4^+ V\beta_1$  T cells, the target cells of MD lymphoma, by programmed cell death (PCD).

These results suggested that the repressive effects on lymphoma formation and nerve enlargement of MD vaccines might be due to decreasing  $CD4^+ V\beta_1$  T cells by PCD, rather than the specific cellular immune responses against MD tumors.