Immuno-PCR/Ag assays. In the nasal secretions, viral DNA was detected in samples with virus titers of $10^{4.3}$ $TCID_{50}$ or more by PCR. On the same samples, Immuno-PCR/Ag remained positive up to day 19 p.i., the last day of test. BHV-1 DNA was detected from the following tissues in all the calves at day 22 p.i. : trigeminal ganglia, ovaries, lungs, nasal and tracheal mucosae, spleen, prescapular and precrural lymph nodes, and PBL, but not skeletal muscles. Virus was not recovered from any of these tissues. The present findings suggest a possible role of leukocytes in BHV-1 latent infection.

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Mechanisms of immuno-suppression induced by Marek's disease virus and involvement of T lymphocytes in the protective effects of Marek's disease vaccine

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Several pathogenic agents, including bacteria, protozoa and viruses, induce some immunomodulations to escape from immune pressure of their hosts. Apoptosis of lymphoid cells is sometimes observed in viral infections, and highly correlated with the viral pathogenesis, especially immuno-suppression. Marek's disease virus (MDV) can suppress cellular immunity followed by development of T-cell lymphoma. Therefore, it has been believed that the immunosuppression plays an important role in lymphoma formation. In this thesis, the author described that MDV induces apoptosis in peripheral $CD4^+$T cells and $CD4^+CD8^+$ immature thymocytes during the latent phase of the infection. Apoptosis in thymocytes was observed at the early stage of the latent phase when thymic atrophy was commonly observed. However, the spleen from MDV-infected chickens showed severe splenomegaly at the same time, and thymocytes did not show apoptotic changes when peripheral $CD4^+$T cells underwent apoptosis. These results suggest that immature thymocytes might be more susceptible to apoptosis than peripheral $CD4^+$T cells, or that the induction mechanism of apoptosis might differ between cell populations.

Down-regulation in the expression of several cellular proteins is one of the immunomodulations induced by pathogenic agents for escaping from the immune pressure of hosts. In the case of MDV-infection, the expression of CD8 molecules on peripheral T cells and thymocytes were down-regulated. CD8 as well as CD4 molecules are necessary for class I or II major histocompatibility complex (MHC)-restricted recognition of foreign antigens, and for thymocyte development. Thus, CD8-down-regulation may be also important for immunosuppression induced by MDV.

MDV-infected chickens have been reported to show functional deficiency in T cells, characterized by delayed rejections of transplantable

tumor cells, decreased mitogenic responses and increased susceptibility to coccidiosis. In order to gain insight into the suppression of cellular immunity, proliferation potentials of $\alpha\beta$ T cells were examined against the stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin. $\alpha\beta$ T cells from MDV-infected chickens did not well incorporate ^{3}H-thymidine in the presence of PMA and ionomycin compared to those from age-matched normal chickens. Furthermore, both $CD4^+$ and $CD8^+$ T cells did not respond to the stimulation, and showed susceptibility to apoptosis even in the presence of PMA and ionomycin. These results indicate that a portion of both $CD4^+$ and $CD8^+$ T cells does not enter the S phase of the cell cycle but undergo apoptosis in the presence of the mitogenic stimulation. Recently, several viruses have been reported to induce down-regulation of Bcl-2 expression, which may correlate with apoptosis caused by viral infections. In MDV-infection, the *bcl-2* gene expression was also down-regulated in T cells, especially $CD8^+$ T cells cultured with PMA and ionomycin. Apoptosis in cultured T cells may result at least partially from the down-regulation of the *bcl-2* gene expression.

In the present paper, the author clarified that MDV accelerates apoptosis of $CD4^+$ T cells and $CD4^+CD8^+$ thymocytes and down-regulate the expression of CD8 molecules on peripheral T cells and thymocytes *ex vivo*. These quantitative and qualitative changes may inhibit smooth immune responses against pathogenic agents. Furthermore, T cells from MDV-infected chickens are susceptible to apoptosis even in the presence of the mitogenic stimulation, suggesting that T cells undergo apoptosis but are not activated even if they encounter the complex of MHC and specific antigen *in vivo*. Thus, these immuno-modulations could highly correlate with the immuno-suppression by MDV.

Lymphoma formation induced by MDV-infection is prevented by neonatal vaccination with an attenuated MDV, strain CVI988. Although $CD8^+$ cytotoxic T cells are assumed to be important for the inhibition of lymphoma development, it is still unclear how the vaccine prevents chickens from this disease. In order to examine the roles of T cell subsets in the anti-tumor mechanisms of the vaccine, T cell subset-deficient chickens vaccinated with strain CVI988 were challenged with an oncogenic MDV, strain Md5. Even in either CD4- or CD8-deficient chickens, the neonatal vaccination prevented chickens from lymphoma formation caused by stain Md5. Interestingly, at the early stage of the latent infection, much higher titers of MDV were recovered from $CD4^+$ T cells, the main target cells for the latent infection and subsequent tumor development, in CD8-deficient vaccinated chickens than in untreated vaccinated chickens. Taken together with these observations, anti-tumor effects of the vaccine are apart from anti-viral immunity by $CD8^+$ T cells because anti-tumor effects of the MD vaccine were still maintained in CD8-deficient chickens.

What is the protection mechanisms of the vaccine? One possibility is that minor cell populations, including natural killer cells, $CD4^-$ $CD8^-$ T cells or $\gamma\delta$ T cells may be responsible for the elimination of MD tumor cells. An alternative possibility of the anti-tumor effects of the MD vaccine may be apoptosis of $CD4^+$ T cells, which could reduce the number of target cells for transformation *in vivo*.

MD vaccines have long been considered as a model of protection immunity against herpesvirus-induced tumor, and the three serotypes of MD vaccines are the only effective vaccine which can prevent infectious lymphomatous disorders among mammalian and avian species. The clarification of both mechanisms of MD pathogenesis and those of anti-tumor effects by MD vaccines can provide many useful information to develop immunotherapy against not only herpesvirus- but

also other virus-induced tumors.

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Study on the analysis of canine renal hemodynamics using Doppler ultrasonography as a non-invasive diagnostic method

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Renal blood flow is closely linked to physiological and pathological changes in the kidney. It is therefore important to examine the blood flow under various conditions. In veterinary medicine, methods available for the estimation of kidney function include blood and blood chemical examinations, urine examination, clearance methods, diagnostic imaging and renal biopsy. Although these methods provide useful information, they also have some clinical limitations such as lack of sensitivity and the complexity or invasiveness of the method. Doppler ultrasonography can be used to estimate renal hemodynamics non-invasively. In veterinary medicine, ultrasonography is an important diagnostic method however, it is mainly used for morphological evaluation. Acute renal failure is one of the diseases encountered frequently in veterinary clinics, and it is important to detect it early in order to be able to provide suitable treatment. The ability to evaluate renal blood flow as a index of renal functions in a clinical setting using Doppler ultrasonography can contribute to the diagnosis, treatment, and prognosis of renal disease.

The purpose of this study is to establish basic data on canine renal hemodynamics using Doppler ultrasonography, and to evaluate the usefulness of Doppler ultrasonography as a new diagnostic method of renal function in comparison with other diagnostic methods under experimentally induced abnormal renal hemodynamics.

1. Estimation of the accuracy of the quantitative measurement of renal blood flow using non-invasive method Doppler ultrasonography. First, a method of approach to the renal artery was determined in order to detect the rate and pattern of the renal blood flow, using 8 adult beagles under general anesthesia. The dogs were placed in dorsal recumbency to visualize the celiac and cranial mesenteric arteries as they branched from the abdominal aorta by scanning the left paramedian in the longitudinal direction. Next, in transverse and coronal sections, the renal artery branching from the abdominal aorta caudal to the cranial mesenteric artery was visualized. Coupled with a color Doppler method, the renal artery was easy to visualize and identify. Second, the renal arterial blood flow was examined using the Doppler system and an electromagnetic flowmeter using 7 mongrel dogs in various experimentally induced hemodynamic states ranging from high to low flow under laparotomy, and evaluated the accuracy of the Doppler system. A significant correlation ($r=$