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## Laboratory of Pathobiology

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The Laboratory of Pathobiology was newly established at the time when the Faculty of Veterinary Medicine was reorganized as the Graduate School of Veterinary Medicine in April 1995. The teaching staff is composed of a professor and an instructor. In addition, an associate professor (*Jun Yasuda*) of the Veterinary Teaching Hospital is concurrently a staff member of this laboratory. Two postgraduate students, three undergraduate students and one research student are enrolled. We teach veterinary clinical pathology and its practice to undergraduate students in the third and fourth years. The staff teaches practical clinical medicine to students at the Veterinary Teaching Hospital. The main research projects in this laboratory are as follows:

1. Veterinary diagnostic ultrasound and its application: Ultrasonography (US) is well established and routinely used in veterinary medicine as a valuable diagnostic imaging modality. However, continuing and more advanced studies are needed to develop US applications from viewpoint of high level diagnosis and to update veterinary medicine. On the other hand, application of US to small experimental animals is not established and little information is available as yet. We are now conducting two projects. We have demonstrated that endoscopic ultrasnography (EUS) is available as an effective diagnostic modality in small animal practice. EUS can provide useful diagnostic information on pancreatic diseases such as atrophic and inflammatory disorders. In addition, gray-scale histogram analysis of EUS images can be employed to evaluate histopathological changes of pancreatic tissues in dogs with experimentally-induced pancreatitis[1]. On the other hand, we have shown the usefulness of US examination for evaluation of drug-induced nephrotoxicity in the rabbits[2] and of parasitic lesions of the liver in rats experimentally infected with *Taenia taeniaeformis*[3].

2. Canine herpesvirus infection: Canine herpesvirus (CHV) causes a fatal hemorrhagic and necrotizing disease in neonatal pups and latent infection in surviving and adult dogs. We have reported that latent CHV infections develop and that the virus may be reactivated, without clinical signs, in dogs with a history of CHV infection[4]. To control CHV infection, the mechanisms of latency and reactivation of the virus should be clarified. We determined the nucleotide sequence of the CHV IE gene, the product of which must play an important role in the latency of the virus. Furthermore, using nested PCR and *in situ* hybridization, we have demonstrated that neurons of the trigeminal and lumbosacral ganglions and lymphocytes of the retropharyngeal and hypogastric lymphonodes are targets of latent infection by CHV.

3. Pathobiological investigation of canine exocrine pancreatic disease: Pancreatic acinar atrophy (PAA) is an important cause of exocrine pancreatic insufficiency (EPI) in the dog. However, the pathogenetic mechanism of PAA is unclear. We have investigated the histopathological changes of the early stage of PAA in a dog. The results suggested the possibility that apoptosis may play a role in the pathogenesis of PAA. Considering the role of apoptosis in the acinar cell disappearance, we are now preparing an experimental study to elucidate the pathogenetic mechanism of PAA. On the other hand, it has been shown that the assay of serum trypsin-like immunoreactivity (TLI) is a highly sensitive and specific test for identification of dogs with EPI including PAA. However, the availability of this test for the diagnosis of pancreatitis is not proved. We are now investigating whether the assay of TLI provides diagnostic indicators of

acute pancratitis.

4. The role of the CD28/CTLA4-B7 pathway in the pathogenesis of autoimmune disease : We studied the role of the CD28/CTLA4-B7 costimulatory T cell activation pathway in the pathogenesis of MRL/lpr mice. Administration of CTLA4IgG inhibited not only autoantibody production and end-organ diseases in the kidney, salivary gland and liver, but also lymphadenopathy. However, lung diseases were not inhibited. To explain the mechanisms of these phenomena, flow cytometric and morphological analyses were performed. It was demonstrated that after CTLA4IgG treatment (i) activation of and IL-4 production from conventional T cells, (ii) production of IFN-$\gamma$ from $CD4^-$ $CD8^-$ $B220^+$ T cells, and (iii) differentiation from activated B lymphocytes to plasma cells were prevented but (iv) development of $CD4^-$ $CD8^-$ $B220^+$ T cells and (v) activation of both B lymphocytes and macrophages were still induced. Thus, we concluded that in MRL/lpr mice the CD28/CTLA4-B7 pathway mediated (i) differentiation of conventional T cells to IL-4-producing Th2/Tc2-like cells, (ii) differentiation of B cells to plasma cells and (iii) IFN-$\gamma$ production from $CD4^-$ $CD8^-$ $B220^+$ T cells, though, (iv) development of $CD4^-$ $CD8^-$ $B220^+$ T cells and (v) activation of both B lymphocytes and a macrophage-containing population were not mediated by the CD28/CTLA4-B7 pathway. Thus, these results emphasize the differential dependence of the CD28/CTLA4-B7 pathway in the pathogenesis of MRL/lpr mice.

5. The development of clinical enzymatic diagnosis : Clinical enzymology is an important clinical diagnostic aid. Quantifying alkaline phosphatase (ALP) isoenzymes in canine serum would provide a useful index in a clinical laboratory. We have reported that combining wheat germ lectin and levamisole inhibition provides adequate separation and quantification of canine ALP isoenzymes[5]. Furthermore, we have demonstrated the mechanism of increase of corticosteroid-induced alkaline phosphatase (CALP) in canine serum and the diagnostic significance of ALP isoenzymes, including CALP, in steroid-induced hepatopathy[6]. On the other hand, we have evaluated adenosine deaminase (ADA) activity and its clinical significance in dogs and cattle[7]. We suggested that ADA analysis may provide one predictive index for tumor behavior.

## References


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