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These results suggest that the animal models may be useful to study the pathogene-

sis of human influenza encephalitis/ encephalopathy.

The original papers of this thesis appeared in *Avian Pathology*, 30 : 269-272 (2001) and *Archives of Virology*, in press (2001).

Studies on the causal agent of so-called fowl glioma

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So-called fowl glioma is characterized by multiple nodular gliomatous growths associated with disseminated nonsuppurative encephalitis. The purpose of the present study was to examine the relationship between so-called fowl glioma and the causative agent. Firstly, to examine the possibility of transmissibility of the causative agents, chicks of Japanese bantams and specific pathogen-free (SPF) chickens (C/O strain White Leghorn) were intracerebrally inoculated with the brain homogenate or culture supernatant from a bantam affected with fowl glioma. All 22 bantams and 16 of 18 chickens in the inoculated groups showed nonsuppurative encephalitis, and the 18 bantams and five chickens developed multiple astrocytic nodules in the cerebrum. These astrocytes immunohistochemically had avian leukosis virus (ALV) antigen. By Southern blot analysis, the ALV proviral sequence was detected both in DNA prepared from the brains of the inoculated birds and in DNA from the inoculum. Ultrastructurally, C-type retroviruses-like particles were detected on the cell surface of the CEF inoculated with brain homogenate of chicken affected with fowl glioma. These results suggested that the gliomatous lesions of the bantams could be transmitted by in-

tracerebral inoculation of the affected tissue and the causal agent was an ALV. Secondly, the various organs as well as central nervous system were examined in SPF chickens inoculated with a brain homogenate from a bantam affected with fowl glioma. Histologically, six of eight inoculated chickens developed nonsuppurative encephalitis in the cerebrum and two of them had the characteristic lesions of fowl glioma. Hyperplastic lymphoid foci concomitantly developed in various organs of these birds, especially in the heart. Apart from these lymphoid foci, nonsuppurative myocarditis was observed in all inoculated birds. Immunohistochemically, the myocardium of all inoculated birds consistently showed strong reactivity for this antigens. These results suggest that the causal virus of fowl glioma has a high propensity to replicate especially in myocardium, and nonsuppurative myocarditis occurs associated with fowl glioma. Finally, to investigate the relationship between the proliferating astrocytes and collagen fibers in the gliomatous nodules, ultrastructural studies were performed on the initial stage of nodules from two bantams affected with naturally occurring fowl glioma. The nodule was composed of closely packed astrocytes. The proliferation of fibroblasts and

relatively abundant collagen fibers were found in and around the adventitia of the blood vessels, which resulted from the invasion of the neoplastic astrocytes into the perivascular tissue. These findings indicated that the small nodules were astrocytoma.

The present studies clarified that so-

called fowl glioma is a viral disease caused by an ALV and that nonsuppurative myocarditis concomitantly occurs in birds affected with the disease. Ultrastructural study indicated that gliomatous nodules at the initial stage of the proliferation should be considered as astrocytoma with reactive perivascular fibrosis.

Original papers of this thesis appeared in *Avian Pathology*, 31 : 197-203 (2002) and *The Journal of Veterinary Medical Science* (2002) (in press).

Vitrification of mouse preantral follicles using a mixture of ethylene glycol and raffinose

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Study of the neuroprotective mechanisms by the spin trap α -phenyl-N-tert-butyl nitron

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α -Phenyl-N-tert-butyl nitron (PBN) is an agent used most widely for investigating free radicals in biological systems and is known to be able to prevent oxidative injury without significant toxicity. PBN attenuates the age-related protein oxidation, decrease of enzyme activity and loss of memory in gerbils, and protects hippocampal cells from ischemia-reperfusion injury. It also protects LEC rats from copper-induced fulminant hepatitis. PBN is thought to exert these biological effects by blocking oxidative stress-induced free

radical reactions. Recently, PBN has the ability to interfere with inflammatory cytokines, to increase anti-inflammatory cytokines and to inhibit the induction of inducible nitric oxide synthase in lipopolysaccharide-administrated rats or mice. These effects may be considered as the interruption of the redox-sensitive inflammatory signaling pathways such as NF κ -B by PBN. Furthermore, PBN attenuated the phosphorylation of p38 mitogen-activated protein kinase (p38) and increased the phosphatase activity in primary rat glia