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Since activated macrophages show enhanced activity of immunological phagocytosis mediated by IgG receptors, the increase of IgG-bound erythrocytes in infected dogs is expected to result in a high degree of erythrocyte loss by erythrophagocytosis, resulting in

severe anemia despite low parasitemia. Thus, it is supposed that activated macrophages play a central role not only in the oxidative damage of erythrocytes but also in the pathogenesis of anemia in dogs infected with *B. gibsoni*.

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Molecular analysis of activating factors for proliferation of JC virus :
influence on JCV promoter by human T-lymphotropic virus type I (HTLV-I) Tax, and
function of JCV agnoprotein

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Polyomavirus JC (JCV) belongs to the polyomaviridae of double stranded DNA viruses which also include simian virus 40 (SV 40) and BK virus. JCV causes the human demyelinating disease, progressive multifocal leukoencephalopathy (PML), which has been observed mainly in immunosuppressive states.

The recent demonstration of cases of PML in association with human T-lymphotropic virus type I (HTLV-I) infection prompted to examine whether the HTLV-I encoded regulatory protein Tax activates JCV transcription. Employing a dual luciferase assay, it was shown that Tax activated the transcriptional potential of JCV promoters only in human neuronal cells *via* NF- κ B pathway, demonstrated by using a JCV promoter which lacks the NF- κ B binding motif, an I κ B α expression vector, and a Tax mutant (M22) lacking the potential for activation *via* NF- κ B pathway. Tax also enhanced the gene expression of JCV T-antigen and VP1. Employing an EMSA, some protein(s) which was exclusive-

ly recognized in non-neuronal cell bound to the NF- κ B motif sequence of JCV, and might inhibit the JCV promoter activity. This study is the first demonstration of the activation of JCV promoter by HTLV-I Tax in a NF- κ B-dependent manner only in neuronal cells, and this transactivation of JCV is regulated by cell-type specific inhibitory protein.

Next, the localization and function of JCV agnoprotein which has not been fully examined, were investigated in JCV-infected cells. Immunocytochemical analysis revealed that agnoprotein was localized in the cytoplasm and perinuclear region of the JCV-infected cells. Mutational analysis of agno-EGFP fusion protein revealed that agnoprotein had a functional nuclear localizing signal (NLS) and nuclear exporting signal (NES), suggesting that the NES function of agnoprotein was predominant, and agnoprotein may translocate to the nucleus caused by some stimulation. Expression of mRNA of the JCV-encoding gene, such as VP1 and large-T was deterio-

rated in agnoprotein-deficient JCV, however, agnoprotein failed to directly transactivate the JCV promoter, suggesting that JCV agno-

protein up-regulates the viral propagation in some way except for viral transactivation.

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Experimental studies on encephalitis/encephalopathy due to virulent influenza virus infection

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In the present study, virulent influenza A viruses were inoculated to the chicken embryos and mice, and the primary target cells of the viruses and the mechanism for the development of encephalitis/encephalopathy were compared between them.

Virulent strains of different pathogenicity (A/tern/South Africa/61 (H5N3), A/whistling swan/Shimane/499/83 (H5N3; 24a5b), A/Hong Kong/483/97 (H5N1), A/Hong Kong/156/97 (H5N1)) or an avirulent (A/duck/Pennsylvania/10128/84 (H5N2)) strain of type A influenza virus were inoculated into the allantoic cavities of chicken embryos. The virulent strains replicated initially in the vascular endothelial cells and then spread to parenchymal cells of the embryos. In contrast, the avirulent virus did not replicate in the vascular endothelial cells. Neither destruction of allantoic membrane nor disseminated intravascular coagulation were found in the embryos infected with the virulent strains. Thus, these findings suggested that the cause of death of chicken embryos was systemic viral infection after initial infection to the vascular endothelial cells.

The invasion routes of the neuropatho-

genic influenza A virus (HK483) into the CNS were investigated. The major pathological findings consisted of bronchitis, bronchopneumonia, ganglionitis, and nonpurulent encephalomyelitis in the brain stem and thoracic spinal cord. Viral antigens were demonstrated in the pterygopalatine, trigeminal and superior ganglia prior to or simultaneously with their detection in the CNS. These findings indicated that the virus reached the CNS through afferent fibers of vagal and/or trigeminal nerves. In the spinal cord, viral antigens were first demonstrated at the anterior part of the thoracic cord, sympathetic trunk ganglia, and spinal ganglia. Therefore, it was suggested that the virus initially replicated in the lungs, and ascended to the thoracic cord via cardiopulmonary splanchnic nerves, sympathetic trunk ganglia and dorsal root ganglia. The antigens were also observed in the olfactory bulb from an early stage of the infection. In this experiment, the invasion routes of the virus into the CNS via the olfactory nerves, cardiopulmonary splanchnic nerves and sympathetic nerves were newly identified in addition to the vagal and glossopharyngeal nerves.