Studies on the mechanism of oxidative damage in erythrocytes of dogs infected with *Babesia gibsoni*

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Although *Babesia gibsoni* cause severe hemolytic anemia in dogs in spite of a low percentage of parasitized erythrocytes in their peripheral blood, the pathogenesis of the anemia remains unclear. Previous studies suggested that increased oxidative damage of erythrocytes in *B. gibsoni*-infected dogs may enhance the phagocytosis of the erythrocytes by macrophages, resulting in severe anemia in infected dogs with a low parasitemia. However, this hypothesis has not been demonstrated, and this study was carried out to elucidate the mechanism underlying the oxidative process in *B. gibsoni*-infected erythrocytes.

In this study, the generation of superoxide anion (O_2^-) and the concentrations of methemoglobin (MetHb) and thiobarbituric acid reactive substances (TBARS) in erythrocytes of dogs infected with *B. gibsoni* were significantly higher than in those of non-infected dogs. When *B. gibsoni* parasites were cultured together with erythrocytes from normal dogs, O_2^- generation of the erythrocytes was significantly higher in the parasitized culture than in the control culture. Increases of MetHb and TBARS concentrations in the parasitized culture were also observed and compared with the control culture. These results suggest that oxidative damage to erythrocytes was induced by the multiplication of *B. gibsoni*, and that parasites may damage not only parasitized erythrocytes but also non-parasitized erythrocytes via the generation of O_2^-. However, this possibility cannot be explained on the basis of the observation that the number of parasitized erythrocytes in peripheral blood in *B. gibsoni*-infected dogs was extremely low. Therefore, in the next experiment we investigated the effects of macrophages on the oxidative damage of erythrocytes in infected dogs.

The peripheral blood monocytes of infected dogs produced a significantly higher chemiluminescent response than those from non-infected dogs when the cells were subjected to non-specific stimulation or specific stimulation. These results indicated that macrophages of *B. gibsoni*-infected dogs were highly activated compared to those of non-infected dogs. Furthermore, the membrane lipid peroxidation of normal dog erythrocytes incubated with activated monocytes from infected dogs was significantly higher than that of erythrocytes incubated with cells from non-infected dogs. These results suggest that the oxidative damage of erythrocytes observed in *B. gibsoni*-infected dogs with a low parasitemia might be induced partly by reactive oxygen species released from the activated macrophages.

The final experiment showed a significant increase of IgG-bound erythrocytes in the peripheral blood of infected dogs compared with erythrocytes in non-infected dogs. In addition, IgG binding to erythrocytes with artificial oxidative damage was significantly higher than that to untreated cells, suggesting that the increase of IgG-bound erythrocytes in infected dogs might reflect the increase of erythrocytes with oxidative damage.

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 virus40 (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-kB pathway, demonstrated by using a JCV promoter which lacks the NF-kB binding motif, an IkBa expression vector, and a Tax mutant (M22) lacking the potential for activation *via* NF-kB pathway. Tax also enhanced the gene expression of JCV T-antigen and VP1. Employing an EMSA, some protein(s) which was exclusively recognized in non-neuronal cell bound to the NF-kB 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-kB-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 VP 1 and large-T was deterio-