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band of the contaminating met Hb, which was estimated from the CO-treated and untreated spectra of the same, hemolyzed sample.

This spectrophotometric method is feasible for the determination of heme content of cyt b$_{558}$ with a small amount of CGD neutrophils in 10–20 ml of blood even in the presence of contaminating Hb.

Cyt b$_{558}$ of human, bovine and porcine have the same absorption peaks in a region. Comparative analysis of cDNA between human and bovine, porcine and murine shows that the highest degree of homology in cyt b$_{558}$. Cyt b$_{558}$ of mammalian species may have the same properties of absorption spectrum. Therefore, this procedure is applicable to another mammalian.

In conclusion, the results of this thesis suggest the MPO-mediated $^{1}\text{O}_2$ generation in neutrophil phagosome and the anti-inflammatory effect of antibiotics. The spectrophotometric determination of cyt b$_{558}$ is applicable to minimal amount of neutrophil from CGD.

The advancement of the research in generation of ROS by neutrophils will be further accelerated by the development of cellular $^{1}\text{O}_2$ detection system. With the realization of such a detection system, the study of generation of $^{1}\text{O}_2$ in vitro will become feasible, and generation of ROS by neutrophils in host defense mechanisms will be cleared.


Pathological studies on distal axonopathy caused by 2,5-hexanedione: comparative study using normal and neurofilament-deficient quail

Takuya HIRAI

Laboratory of Comparative Pathology,
Department of Veterinary Clinical Sciences,
Graduate School of Veterinary Medicine,
Hokkaido University, Sapporo 060–0818, Japan

2,5-hexanedione (2,5-HD) is the common $\gamma$-diketone metabolite of neurotoxic chemicals such as n-hexane or methyl n-butyl ketone, and has been widely utilized as the most convenient compound for experimental studies of $\gamma$-diketone neuropathy. Traditionally, $\gamma$-diketone neuropathy is classified as a distal axonopathy, which is characterized by distal axonal swellings with neurofilament (NF) accumulation and degeneration in long tracts of the central nervous system (CNS) and long nerves of the peripheral nervous system (PNS). The relationship between NF accumulation and axonal degeneration, however, has not been adequately elucidated in this toxic neuropathy. In the present study, this relationship was examined using normal and neurofilament-deficient (Quv) quail.

Both normal and Quv quail were inoculated intraperitoneally with 350 mg/kg per day 2,5-HD for 6 consecutive weeks. 2,5-HD induced distal axonopathy in about 4–6 weeks in normal quail and acute neurotoxicity in Quv quail. Although all treated Quv quail showed neurological signs, there were no recognizable 2,5-HD-induced lesions in the nervous system. Two explanations for the absence of the distal axonopathy in Quv quail treated with 2,5-HD are possible. The development of axonopathy may require an accu-
mulation of NFs in the distal part of the axons and Quv quail lacking NFs may not be affected with the distal axonopathy. Alternatively, because Quv quail died or were euthanatized after a short treatment period, acute neurotoxicity may not have had time to develop.

To investigate the possibility that the absence of the distal axonopathy in Quv quail might be due to the shortness of the treatment period, a lower dose of 2,5-HD (175 mg/kg per day) was administered for a long term (24 weeks) to normal and Quv quail. Some treated normal quail had central-peripheral distal axonopathy. In contrast, distal axonal degeneration did not appear in any Quv quail. These results indicated that distal axonal degeneration did not occur without NF accumulation. In conclusion, NF accumulation is an essential factor in the development of distal axonopathy in γ-diketone neuropathy.


Studies on latent infection, and immediate early and early protein gene of canine herpesvirus

Masahiro Miyoshi

Laboratory of Pathobiology
Graduate School of Veterinary Medicine,
Hokkaido University, Sapporo 060-0818, Japan

Canine herpesvirus (CHV), belonging to the subfamily Alphaherpesvirinae, causes a fatal hemorrhagic disease in neonatal pups, and usually subclinical infection in the respiratory and genital tracts in adult dogs. The virus remains latent in convalescent dogs and stress or immunosuppression leads the virus to reactivate. Asymptomatic excretion of the reactivated CHV usually occurs and pose risks for transmission of the virus, causing neonatal mortality in breeding kennels and the spread of CHV infection. It is, therefore, important to clarify the mechanisms of latency and reactivation of the virus.

To provide information on the latency and reactivation of CHV, the virus was inoculated into female adult dogs via different routes. In situ hybridization analyses on tissues of the convalescent dogs revealed that the latent CHV harbored in the nuclei of the trigeminal ganglionic neurons and the retropharyngeal lymphocytes. Northern blot hybridization and reverse transcription-PCR analyses on the latently infected tissues demonstrated that the latency associated transcript (LAT) of CHV was an approximately 6kb RNA and generated as an antisense transcript to the immediate early (IE) and early (E) genes of the virus. Thus, the CHV LAT was suggested to be involved in the latency and reactivation.

To define the structure of the CHV IE and E proteins, the inverted repeat region and its vicinity linked to the unique long region of the genome were cloned and sequenced. The IE protein was predicted to consist of 1,383 amino acids. The amino acid sequence suggested that CHV IE protein was a homologue of the infected cell protein 4 (ICP4) of herpes simplex virus 1 (HSV-1) which played an important role in viral gene expression as a transactivator. The gene flanking downstream the IE protein gene was transcribed in the E phase and encoded the ICP0