PATHOLOGICAL STUDIES ON THE RABID DOGS AND MICE EXPERIMENTALLY INFECTED WITH RABIES VIRUS

狂犬病発病犬および狂犬病ウイルスに感染したマウスに関する病理学的研究

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Rabies is a highly fatal zoonotic disease caused by rabies virus. It is remains a serious global public health problem and causes more than 55,000 human deaths annually, particularly in Asia and Africa. In endemic countries, domestic dogs remain the major reservoir and vector for rabies virus infection and play an important role in transmission of rabies virus to humans. Viral transmission is achieved through contact with the virus contained in the saliva of an infected animal, often through biting. After biting by an infected animal, the rabies virus binds to the nicotinic acetylcholine receptors at the neuromuscular junction in the muscle fiber. The virus enters through the peripheral nerves and reaches the central nervous system (CNS) by centripetal spread. The virus then spreads centrifugally along the autonomic and sensory nerves to the peripheral non-nervous tissues, including the salivary glands, muzzle skin, adrenal glands, gastrointestinal tract, pancreas and heart.

However, the mode of centrifugal spread of the virus and pathological findings in the non-nervous tissues has not been fully understood. The purpose of my study was to investigate the pathological changes in the peripheral non-nervous tissues of head using naturally infected rabid dogs and experimental models. In addition, to obtain the more information about neuropathogenesis of rabies in mice, street rabies virus (1088-N0 strain) was inoculated into the right hind limb of *dd*Y mice, and the primary target cells and the sequential involvement during infection in the peripheral tissues and CNS were investigated.

(1) A pathological study of the salivary glands of rabid dogs in the Philippines

Salivary gland infection is important in natural rabies vectors because the rabies virus is excreted in the saliva, which allows transmission to additional host by biting. However, detailed pathological findings in salivary glands and analysis of the excretion mechanism remain unclear.

Here, I first investigated the histopathology and analyzed the mechanism of excretion into the oral cavity. Mandibular and parotid glands of 22 rabid dogs and three control dogs were used.

Mild to moderate non-suppurative sialadenitis was observed in the mandibular glands of 19 of the 22 dogs, characterized by loss of acinar epithelium and infiltration by lymphoplasmacytic cells. Viral antigens were detected in the mucous acinar epithelium, ganglion neurons, and myoepithelium. Acinar epithelium and lymphocytes were positive for anti-caspase-3 antibodies and TUNEL staining. In contrast, no notable findings were observed in the ductal epithelial cells and serous demilune. In the parotid gland, the acinar cells, myoepithelium, and ductal epithelium all tested negative. These findings confirmed the path through which the rabies virus descends along the facial nerve after proliferation in the brain to reach the ganglion neurons of the mandibular gland, subsequently traveling to the acinar epithelium via the salivary myoepithelium. Furthermore, the observation that nerve endings passing through the myoepithelium were absent from the ductal system suggested that viral proliferation and cytotoxicity could not occur there, ensuring that secretions containing the virus are efficiently excreted into the oral cavity.

(2) Localization of the rabies virus antigens and diagnostic utility of the muzzle skin of rabid dogs

The early diagnosis of rabies in animals is essential for the prevention of exposures of health care workers and for initiation of specific therapy if an aggressive approach is considered. The most commonly used method is the direct fluorescent antibody test using fresh brain samples. However, this method is laborious, time-consuming and there is a high risk of exposure to the rabies virus. Therefore, alternative methods for rabies diagnosis are required based on a simple collection of non-neural specimens. Here, I further evaluated the diagnostic utility of the muzzle skins containing follicle-sinus complexes (FSCs) and localization of the viral antigens in FSCs of rabid dogs in the Philippines. The muzzle skins containing FSCs of 9 rabid dogs (3 euthanasia, 6 found dead) and three control dogs were used.

Immunohistochemistry and immunofluorescent antibody test analyses confirmed the presence of viral antigen in the muzzle skins in all rabid dogs (100% specificity). Most of viral antigens in the FSCs were detected in the cytokeratin 20 and CAM 5.2 positive Merkel cells.

These results confirmed the rabies virus antigen was localized in the Merkel cells, which were an important mechanosensory receptor of the tactile hair, and also the path through which the rabies descends along the trigeminal nerve after proliferation in the brain to reach the Merkel cell of the tactile hair. Furthermore, infection of rabies virus in the trigeminal tract and Merkel cells might be responsible for reduction or loss of skin sensation. Therefore, it was suggested that muzzle skin are very useful as alternative source of postmortem diagnosis of rabies, especially in rabies-endemic developing countries.

(3) Comparative pathology about peripheral tissues of mice intramuscularly infected with fixed (CVS-11) and street (1088) rabies strains

Rabies virus can cause fatal encephalomyelitis, but the involvement of peripheral nervous and non-nervous tissues of the head has not been well characterized. In this study, the histopathological changes and the distribution of viral antigens in peripheral nervous and non-nervous tissues of the head were investigated in adult C57BL/6J and *dd*Y mice that were infected intramuscularly with fixed rabies virus (CVS-11 strain) and street rabies virus (1088-N0 and 1088-N30 strains), respectively.

Mice infected with the CVS-11 and 1088-N0 viruses showed paralysis at 5 DPI and died, while mice infected with 1088-N30 virus recovered from disease. Histopathologically, mild to moderate encephalomyelitis was observed in all infected mice, however, the lesions in the non-nervous peripheral tissues different among the strains. Viral antigens were detected in the trigeminal ganglia, trigeminal nerve, maxillary nerve, infraorbital nerve, hypoglossal nerve, retina, lingual mucosa, taste cells in the circumvallate papillae, lingual minor salivary glands, facial muscle, and muzzle skin of mice infected with 1088-N0. On the other hand, the 1088-N30 viruses had infected the trigeminal ganglia, trigeminal nerve, maxillary nerve, hypoglossal nerve, lingual mucosa, lingual minor salivary glands, and muzzle skin. The CVS-11 viruses had restrictively infected the trigeminal ganglia, trigeminal nerve, lingual mucosa and the retina. The number of inflammatory cells and titer of viral neutralizing antibodies (VNA) were high in 1088-N30. These findings demonstrate that, although three strains of rabies virus grew in the CNS, the street strains spread rapidly to the peripheral nervous tissues and peripheral non-nervous tissues of the head. In addition, early induction of inflammation and VNA are an important role to prevent the virus spread from the CNS to peripheral tissues.

(4) Pathological study on the central nervous system and peripheral tissues of *dd*Y mice intramuscularly infected with street rabies virus (1088-N0 strain)

Street virus 1088 strain isolated from a woodchuck in the Centers for Disease Control in Atlanta, USA. This virus shows strong neurotropism and kills the mouse by hindlimb inoculation with wide distribution of virus antigens in the brain. It was proposed that the number of *N*-glycosylation sites on the G protein was one of the determinants of the 1088 strain pathogenicity in mice. However, the detailed pathological findings of 1088 strain in the mice still not published. The purpose of the present study was to clarify the histopathological findings produced in mice by inoculation with street rabies virus, 1088 strain.

*dd*Y mice was inoculated into the right hindlimb with street rabies virus (1088-N0) and pathological changes in the CNS and peripheral tissues were studied. At 5, 8, 11 days post-inoculation (DPI), five mice per a group were sacrificed and their sera, and brain, spinal cord, and muscle were sampled. Hind limb paralysis was observed at 5 DPI and progressed to quadriparalysis at 8 DPI. From 5 DPI, degeneration of neurons in the spinal dorsal ganglia was observed. At 8 DPI, the number of degenerated ganglion neurons increased and some axon exhibited vacuolated changes. At 5 DPI, the viral antigen was found in the hindlimb muscle, spinal dorsal ganglia, motor neurons in the ventral horns, red nuclei, medulla oblongata and cerebral cortex (M1 motor area). From 8 DPI, the viral antigen was widely distributed throughout the brain. The number of T lymphocytes, microglial cells, astrocytes and viral neutralizing titers were increased as the infection progressed. These results suggested that 1088 virus ascended the spinal cord via mainly afferent fibers at early stage of infection and move to cerebral cortex using descending spinal tract. In addition, the pathological changes are more severe in the spinal dorsal gangliocytes than those found in the nerve cells of the brain and spinal cord. Therefore, it was suggested that the possibility that the selective vulnerability of spinal dorsal gangliocytes and their nerve fibers in mice infected with 1088 strain deeply participated in clinical signs.