Encephalitis in Mice Inoculated Intranasally with an Influenza Virus Strain Originated from a Water Bird

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Although laboratory mice are not naturally infected with influenza virus, intracerebral inoculation with mouse-adapted human influenza viruses or reassortants of human and fowl plague viruses caused encephalitis in mice [11, 13]. On the other hand, intranasal inoculation with avian influenza viruses has not resulted in encephalitis in mice. In the present study, marked neuropathological lesions were induced in mice by intranasal inoculation with an avian influenza virus strain derived from a water bird.

The original virus, A/whistling swan/Shimane/499/83 (H5N3) (strain 499) was of low virulence for chickens [8, 10]. This virus was passaged in the brain of chicks five times after 24 serial passages in the air sac of chicks (strain 24a5b). The strain 24a5b produced 100% mortality in chicks [12]. Virulence of the 24a5b was compared with the original virus passaged in embryonated eggs four times (4e).

Forty-two, 5-week-old ddY mice were allotted to 3 groups: 24 mice for the 24a5b strain; 15 mice for the 4e strain; and 3 mice for uninoculated control. Each group was housed separately and allowed free access to food and water. Three mice each from the 24a5b group were necropsied at days 1–5, 7, 10 and 14 post-inoculation (PI); three mice each from the 4e group were at days 1, 3, 7, 10 and 14 PI, and one mouse each from the control group was at days 1, 7 and 14 PI. This experiment was performed in the sealed room.

The liver, spleen, kidneys, heart, lungs and brain were collected from each mouse for histopathological and immunohistological examinations. Tissue samples were fixed in 10% formalin (pH 7.2), embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). For immunohistological examination, the labelled streptavidin-biotin (LSAB) method was done. Other paraffin sections were placed on neoprene coated glass slides. These slides were deparaffinized, digested with trypsin, and endogenous peroxidase activity was quenched with 3% H2O2 in distilled water. After blocking of nonspecific reactions with normal swine serum, the sections were incubated overnight with rabbit anti-strain 499 hyperimmune serum at a 1:1,000 dilution at 4°C. Following a 5-min wash in phosphate buffered saline (PBS, pH 7.2), they were covered with biotinylated swine anti-rabbit IgG antibody (DAKO, Glostrup, Denmark) at room temperature for 1 hr. This was followed by a 5-min wash in PBS and 1 hr incubation at room temperature with peroxidase-conjugated streptavidin (DAKO). Specific reaction was visualized with diaminobenzidine and hydrogen peroxide.

Virus recovery from the brain of the mice inoculated with the 24a5b was done at each occasion of necropsy by the method described previously [9].

Clinical findings: In the 4e and control groups, none of the mice showed clinical signs, and no deaths were recorded during the experimental period. In the 24a5b group, two mice died and one was killed at a moribund state at day 7 PI.

Histological and immunohistological findings: In the 24a5b group, one of three mice killed at day 5 PI and all the three mice necropsied at day 7 PI had mild to severe nonsuppurative encephalitis located mainly in the pons and medulla oblongata. The lesion consisted of scattered foci of necrosis, glial reactions (appearance of rod cells and swelling of astrocytes), and perivascular cuffings of mononuclear cells and some neutrophils (Fig. 1). Rod cells and mild perivascular cuffings were also found in the cerebellar nuclei. Nerve cells, glial cells and a few vascular endothelial cells in the lesions reacted positively with anti-strain 499 serum (Fig. 2). The positive findings were observed mainly in the facial motor nucleus, vestibular nucleus, reticular nucleus, and lateral and medial cerebellar nucleus (Fig. 3). At day 14 PI, all mice in the 24a5b group showed mild interstitial pneumonia, but viral antigen was not detected in the lesion. Neither histological nor immunohistological findings were found in other organs of the 24a5b group. In the 4e group, mild to severe broncho-interstitial pneumonia was observed at day 7 PI, and necrotic bronchial epithelial cells reacted positively with the anti-strain 499 serum. Histological and immunohistological findings in other organs were not evident in the 4e group.
In control mice, no significant lesion was observed.

**Virus recovery from the brain:** The virus with titers of $10^{12}-10^{6.9}$ EID$_{50}$/g tissue was isolated from the mice of the 24a5b group at days 4, 5 and 7 PI. Virus was not isolated thereafter.

Natural infection of influenza virus or influenza encephalitis has not been reported in mice [2]. It has been shown that genetic reassortant [11] or mouse-adaptation with intracerebral inoculation [2, 13] was required for influenza viruses to replicate in the central nervous system (CNS) of mice. In the present study, the mice intranasally inoculated with an avian influenza virus which was highly pathogenic for chicken developed encephalitis. This is thus the first finding that avian influenza virus caused encephalitis in mice by intranasal inoculation. The strain 4e did not show such neurovirulence. Genomic comparison of the strains 24a5b
with the 4e revealed multiple mutations in the HA gene (unpublished data). A viral genetic basis on the acquisition of the enhanced neuropathogenicity for mice remains to be elucidated. Some avian influenza viruses have caused sporadic and self-limiting infections in mammals, such as seals, minks, whales, and humans [2, 3–5, 14]. This report revealed that an avian influenza virus which is highly pathogenic for chickens infects mice and causes severe encephalitis by intranasal exposure.

Experimental viemetic exposure to neurotropic influenza virus has produced panencephalitis, meningo- or ependymoencephalitis in birds and mice [1, 6, 7, 11]. In the present study, CNS lesions were mainly located in the pons, medulla oblongata, and cerebellar nuclei. Transneuronal infection of influenza virus via the olfactory and trigeminal nerve fibers to the CNS has been reported with a recombinant virus; the lesions induced by the virus were restricted to the brain stem, cerebellum, pons, reticular formation, posterior colliculi, mammillary body and basal part of the frontal cortex [11]. Therefore, the cranial nerves may also be important as one of invasive routes to the CNS.

REFERENCES