Original Article

Comparative Analysis of the Pathogenic Mechanisms of Street Rabies Virus Strains with Different Virulence Levels*

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Abstract

Objective To characterize two strains of street rabies virus (RABV) isolated from the brain tissue of cattle from Inner Mongolia. Differences in the histopathological and ultrastructural changes in the brain tissue of infected mice were determined to reveal variation in the pathogenesis of infection between street rabies virus strains.

Methods Ten-day-old mice were intracranially inoculated with one of three virus strains and brain tissue harvested when the mice were moribund. Various histopathological and ultrastructural markers of disease were then compared between the groups.

Results Infection with the street virus strain CNM1101C resulted in severe neuronal dendrites damage, but only mild cell apoptosis, T lymphocyte infiltration and microglial activation. Infection with the other street virus strain, CNM1103C, was characterized by cell apoptosis, T lymphocyte infiltration and microglial activation as well as dendrites damage. However, in comparison, infection with the attenuated virus strain CTN caused severe T lymphocyte infiltration, microglial activation and cell apoptosis, but left the neuronal dendrites intact.

Conclusion The two street rabies virus strains isolated from cattle from Inner Mongolia had different levels of virulence and caused distinct pathological changes in infected mice. Therefore, we concluded that different pathogenic mechanisms exist between different RABV strains.

Key words: Immunohistochemistry; Rabies virus; Apoptosis; Pathogenicity

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INTRODUCTION

The rabies virus causes a fatal disease of the nervous system in humans and animals[1,2-4]. As a result of large-scale canine vaccination initiative, human rabies cases in Canada and the United States have decreased substantially since the 1940's[1-2,4-5]. However, in developing countries, the mortality rate of human rabies remains very high[1-2]. Every year, more than

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50,000 people and millions of animals worldwide die of rabies, and nearly 10 million people receive rabies prevention therapy after exposure to the virus[2-3]. Thus, rabies infection continues to pose a grave threat to human and animal health.

Despite years of research, the mechanism of nervous system dysfunction in rabies cases remains unclear. Studies have found that the virus causes abnormalities in the cerebral arteriovenous current, ion channels, and neurotransmitters after infecting nerve cells[6-8]. However, these abnormalities are not present in all infected animals and do not fully explain the pathogenic mechanism of rabies[9-16]. Our current understanding of the pathogenicity of the rabies virus has mostly come from infection of experimental animals with fixed strains of the rabies virus[17-25]. However, the pathological changes observed in these experimental infection models greatly differ between the various virus strains studied[1,17]. Moreover, infection with fixed strains in the laboratory does not fully reflect the pathogenetic process in animals infected with wild-type or street rabies virus under natural conditions.

Therefore, to explore the pathogenesis of street rabies virus, we compared the histopathological and ultrastructural characteristics of two street rabies virus strains, which have greatly disparate biological characteristics and virulence levels, with an attenuated rabies virus strain in mice[26-27].

MATERIALS AND METHODS

Viruses

The rabies virus strains CNM1101C and CNM1103C were isolated from cattle from Inner Mongolia in 2011. The attenuated rabies virus strain CTN was kindly provided by the National institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The parental virus of this strain was isolated from the brain tissue in a rabies patient in Zibo City, Shandong Province in 1956, and was passaged in human diploid cells for 50 generations followed by consecutive passages in mouse brain for 56 generations. The resultant attenuated rabies virus strain[1] was approved by the World Health Organization (WHO) in 1983 and 2005 for the production of rabies vaccine in China[17].

Animals and Sample Preparation

All animals experiments described in this paper have been conducted according to the Guideline on the Humane Treatment of Laboratory Animals stipulated by the Ministry of Science and Technology of the People's Republic of China (MOST) and approved by the Animal Welfare Committee of the Military Veterinary Research Institute, Changchun, China. All animals were housed in a climate-controlled laboratory with a 12 h day/ 12 h night cycle. No human patient derived clinical materials and non-human primates were used in the completion of these studies.

ICR (Institute of Cancer Research) mice of different ages (1 d, 4 d, 10 d, 2 weeks, 3 weeks, 4 weeks) were purchased from the Institute, of Laboratory Animal Medical Sciences (CAMS & PUMC, China). The 1-day-old mice were intracranially inoculated with strains CNM1101C, CNM1103C, or CTN (n=10 for each strain). When the mice were moribund, brain tissue was harvested and used to make 10% tissue suspensions. The virus titer of the 10% tissue suspension was determined using the Reed-Muench method. Next, 1000 times of the intracerebral median lethal dose ($ICLD_{50}$) of each virus strain suspension was inoculated into ICR mice (n=4 for each strain) of different ages via the intracranial or intramuscular (hind leg) routes, and the mice were continuously observed for morbidity[17,19].

When these mice were moribund, they were anesthetized with an intraperitoneal injection of 0.2 mL of 10% chloral hydrate. The chest cavity was then opened to expose the heart and a perfusion needle was inserted through the tip of the left ventricle. The mouse was then rapidly perfused with 3-5 mL phosphate-buffered saline (PBS), followed by 6-10 mL 4% paraformaldehyde precooled to 4 °C for fixation. Brain tissue was then embedded in paraffin wax and sectioned onto poly-L-lysine coated slides.

Examination of Mouse Brain Tissue

Direct Immunofluorescence Staining of Rabies Virus-specific Antigen The mouse brain tissue specimens were subjected to direct immunofluorescence assay (DFA). The nucleocapsid protein of rabies virus was detected with a fluorescein-labeled monoclonal antibody[28] (Rabies DFA Reagent, CHEMICON).

Electron Microscopy Mouse cerebellar tissue specimens were fixed in 2% glutaraldehyde solution supplemented with 2.5% paraformaldehyde and stored at 4 °C. Samples were post-fixed in 1% osmium tetroxide, followed by dehydration and