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Neuropathogenesis of Japanese encephalitis virus

Kotaro Yasui

Department of Microbiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan

In the central nervous system, the Japanese encephalitis virus can replicate only in neurons. The mechanism of the type of neurotropism was analyzed. The susceptibility to Japanese encephalitis virus infection in the rat brain was closely associated with neuronal immaturity. The initial specific binding of the virus to cells is one of the reasons for neurotropism of the Japanese encephalitis virus. The treatment of Japanese encephalitis virus infection with the neutralizing monoclonal antibody against the E protein did not inhibit the virus from binding to the cell surfaces, but strongly inhibited Japanese encephalitis virus–induced cell fusion and internalization of the virus into the host cells. One of the genome regions responsible for neuropathogenesis of the Japanese encephalitis virus was located on the E protein–coding region. The 138th amino acid of the E protein was important for neuropathogenesis expression of the Japanese encephalitis virus. The cell fusion activity of the E protein was closely correlated with neuropathogenesis of the virus. *Journal of NeuroVirology* (2002) **8(suppl. 2),** 112–114.

Keywords: Japanese encephalitis virus; neuropathogenesity; neurotropism

Introduction

Japanese encephalitis virus (JEV) is prevalent in Asian monsoon areas. The distribution is gradually expanding. Recently, more than 35,000 cases are reported every year. JEV causes serious encephalitis when the virus replicates in central nervous system (CNS) because replication occurs only in the neurons. An analysis of the mechanism inducing neurotropism of JEV is discussed in the article.

Results and discussion

Analysis of the neurotropism of JEV using developing rat brains

JEV was inoculated in the brains of young. After 3 days, the infected brains were processed by immunohistochemical staining. The neurons of the substantia nigra, caudate putamen, and thalamus were most sensitive to JEV infection. These findings are similar to reported cases in humans.

It is widely accepted that in the development of the cerebral cortex, successively generated neurons in fetal life migrate outward and come to lie at a more superficial level. This sequence of events is known as the "inside-out" rule. At the age of 4 days, diffuse infection involving all neurons in the cortex was observed. At the age of 6 days, although the neurons in the upper layers still contained the viral antigens, the deeper layers were not infected, with the exception of those neurons attached to the basement membrane. On the 8th day, neurons in the superficial position of layer II were stained, with the remaining neurons negative. The results suggest that immature neurons are more susceptible to JEV infection than mature neurons.

To confirm this result, we conducted neuronal transplantation experiments. Fast blue–labeled neurons, taken from an embryonic cortex on the 19th day of gestation, were transplanted into the brain of 15-day-old rats followed by JEV infection 3 days after neuron transplantation. JEV infected only the immature neurons labeled with fast blue. In contrast, for rats infected with JEV at 9 days post neuron transplantation, no detectable viral antigen was present (Ogata *et al*, 1991).

Address correspondence to Kotaro Yasui, Department of Microbiology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai Fuchu, Tokyo 183-8526, Japan. E-mail: yasui@tmin.ac.jp

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Further experiments were conducted on the primary culture system using the rat brain cerebral cortex. JEV was introduced in the primary cortex culture at different intervals beginning on the 8th day and concluding on the 25th day. After 2 days, the cells were stained immunocytochemically. JEV antigens were observed in microtubule-associated protein (MAP)2-positive neurons but not in neuroglia cells after 12 days in the culture. JEV could infect more than 65% of the neurons after 8 to 15 days in culture. These neurons had the characteristic morphology of immature neurons. However, after 21 days in the culture, most neurons, which were electrophysiologically active and morphologically mature, were not infected.

To investigate the basis of the neurotropism of JEV, the process by which the virus binds to neural cells was examined. After the culture cells were infected with highly concentrated JEV at 4°C, the cells were treated with anti-JEV antibodies and stained immunocytochemically. Strong binding of JEV to neurons at 12 days in culture was observed. On day 25, only low binding occurred (Kimura-Kuroda *et al*, 1992).

We concluded in this section that the susceptibility to JEV infection in rat brains is closely associated with neuronal immaturity. Thus, developing neurons are the major target of JEV. The initial specific binding of the virus to cells may contribute to neurotropism of JEV.

Analysis of neutralizing mechanism of JEV with antibodies for elucidation of infection mechanism of JEV

A neutralizing monoclonal antibody (Mab) 503 and non-neutralizing Mab 204 for control against the E protein of JEV were used in this experiment. The neutralizing Mab 503 did not inhibit JEV binding to host cells. After JEV (multiplicity of infection [m.o.i.] = 1800)-Mab mixtures were adsorbed to vero cells at 4°C, the temperature was increased to 37°C. The JEV on the surface of vero cells was stained cytochemically and observed by confocal laser microscopy. The neutralizing Mab 503 treatment strongly inhibited JEV internalization into vero cells, but non-neutralizing Mab 204 treatment did not inhibit internalization.

Therefore, JEV can induce cell fusion. Vero cells were infected with JEV at m.o.i. 10 and cultured for 2 days. After the culture pH reached 5.5, the cells were incubated with Mab 503 or Mab 204. The neutralizing Mab 503 inhibited JEV-induced cell fusion completely but the non-neutralizing Mab 204 did not (Butrapet *et al*, 1998).

In conclusion, treatment of JEV with neutralizing Mab 503 did not inhibit the virus binding to the cell surface, but it strongly inhibited JEV-induced cell fusion and internalization of JEV into the host cells.

Development of postencephalitic parkinsonism model on rats

Substantia nigra neurons are very sensitive to JEV infection, and the neurons are destroyed by JEV replication. From these findings, we developed a postencephalitic parkinsonism model on rats. JEV was infected at age of 13 days and the rats were observed for 12 weeks. The dopamine level of brain striatum was measured and motor activity was examined by applying a pole test, which is used to evaluate quantitative bradykinesia in rats. The Dopamine levels of JEV-infected rats was greatly decreased and time required for the completion of the pole test of JEV-infected rats was significantly increased. However, the dopamine level of brain striatum in JEV-infected rats treated with a thyrotropin-releasing hormone (TRH) injection improved significantly. Furthermore, TRH injection also improved bradykinesia in JEV-infected parkinsonism rats (Ogata *et al*, 1998). Thus, a JEV-induced postencephalitic parkinsonism model using rats could be developed. In short, TRH treatment improved bradykinesia in JEV-induced parkinsonism rats.

Analysis of neuropathogenesity of JEV using virulent, avirulent, and avirulent revertant virus

An at222 virus is an attenuated virus derived from virulent AT31 virus of JEV after propagation on hamster kidney cells. The virus had been used for vaccination of pigs in Japan. Revertant virus is a neurovirulent revertant derived from at222 virus propagated on C6/36 mosquito cells and mouse brains. SA14 virus is a wild-type virus. SA14-14-2 virus is an attenuated virus derived from SA14 virulent virus. The virus has been used as a live vaccine for human in China. JaOArS982, JaGAr-01, and Beijing-1 are wild-type viruses. The base sequences of whole genome of those viruses were compared each other. And common rules for attenuation were analyzed.

The neurovirulent activities of AT31, at222, and avirulent revertant virus were tested by using mice. AT31 virus killed all mice when one plaque-forming unit (PFU) virus was inoculated through the intracranial (IC) route; however, at222 virus could not kill even when 10^5 PFU virus was inoculated intracranially. But the revertant virus recovered significantly neurovirulent characteristics. LD_{50} of the revertant virus was about 10 PFU.

There was no common rule in whole genome for attenuation except E protein–coding region. The 138th amino acid position of the E protein of wild-type JEVs were glutamic acid; however, those of attenuated viruses were lysine. Interestingly, the 138th amino acid of the revertant virus reverted to glutamic acid, but the base sequence of other areas of the revertant were completely the same as that of the at222 virus. The AT31 virus had

strong cell fusion activity, but the cell fusion activity of the at222 virus was very weak. However, the revertant virus restored cell fusion activity significantly.

Based on these results, it can be concluded that one of the genome regions responsible for neuropathogenesis of JEV is located on the E protein–coding region. The 138th amino acid of the E protein is important for neuropathogenesis expression of JEV[1]. The cell fusion activity of the E protein is closely correlated with neuropathogenesis of JEV[1] (Li *et al*, unpublished data).

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[1]In the CNS, JEV can replicate in only neurons. The mechanism of this strict neurotropism was analyzed. The susceptibility to JEV infection in the rat brain was closely associated with neuronal immaturity. The initial specific binding of the virus to the cells was one of the reasons for the neurotropism of JEV. Treatment of JEV with the neutralizing monoclonal antibody against the E protein of JEV did not inhibit the virus binding to the cell surface, but strongly inhibited JEV-induced cell fusion and internalization of JEV into the host cells. One of the genome regions responsible for neuropathogenesis of JEV was located on the E protein–coding region. Amino acid 138 of the E protein was important for neuropathogenesis expression of JEV. The cell fusion activity of the E protein was closely correlated with neuropathogenesis of JEV.